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TRANSFAUNATION AND REPAIR OF DAMAGE IN THE RAT TAPEWORM, *HYMENOLEPIS DIMINUTA**

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The successful *in vitro* cultivation of many parasitic helminths has resisted, to date, the best efforts of parasitologists partly because their normal *in vivo* requirements are inadequately known. Much is known, of course, concerning the physiology of normal parasitic sites, but perhaps more can be learned by altering these sites and testing the adaptability of parasites placed in them.

This paper is the first of a series dealing with results of infections of *Hymenolepis diminuta* in altered hosts. It is concerned 1) with proof that surgical transfaunation is a successful method of achieving infections, and 2) with results of experiments in which tapeworms have been implanted after mutilations to test their powers of repair or regeneration. Subsequent reports will discuss the effects on tapeworms implanted in hosts altered in other ways.

MATERIALS AND METHODS

Wistar rats and a strain of *Hymenolepis diminuta* originally obtained from the Rice Institute, Houston, Texas, have been used in these studies. Rats were bred and maintained on Purina Laboratory Checkers in our laboratory. Tapeworms have been cultivated in rats and in the confused flour beetle, *Tribolium confusum*, following somewhat modified routine methods. The modification which yielded large numbers of pure *H. diminuta* eggs is as follows: a day's collection of rat fecal pellets containing eggs is mixed for 1 minute in 60 ml of water in a Waring Blendor equipped with a semi-micro container. The mixture is strained through cheesecloth into two 250-ml sedimentation glasses; additional water is poured through the cheesecloth to fill both glasses which are then allowed to stand undisturbed for 20 or more minutes. The supernatant is discarded and the washing repeated until the discarded supernatant is clear. Additional water is then added together with approximately 90 grams of household salt which is thoroughly mixed with the fecal solution. After standing about 10 minutes the top layer is transferred to 50-ml centrifuge tubes containing about 5 grams of salt and is mixed again. Following centrifugation the top 10 ml is transferred to 15-ml centrifuge tubes and is carefully overlain with fresh water. These tubes are centrifuged and the concentrated eggs form a narrow golden band in the interphase. The eggs are transferred to other 15-ml tubes, washed and centrifuged several times with water. Finally the eggs are lifted from the bottom of the tubes, mixed with powdered oatmeal which has been shaken through a sieve having 40 openings per linear inch, placed on small pieces of paper toweling and introduced into petri dishes containing flour beetles. The beetles are usually starved for 2 or 3 days prior to being fed and are re-fed fresh egg-oatmeal mixture daily for 5 days. Heavy and consistent cysticercoid infections, reaching counts as high as 30–40 ripe larvae per beetle, have been routinely achieved by this method.

For implantation of normal and mutilated tapeworms, rats were lightly etherized, clipped and swabbed with 70% ethanol on the right flank. A small incision, through skin and muscle,

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was made on the flank and the small or large intestine pulled to the outside. A small nick was made in the intestine and the tapeworm placed into the lumen by means of a fine polyethylene tube after which the intestinal opening was closed with 6-o braided silk sutures. Muscle layers were rejoined with 4-o braided silk sutures; the skin was rejoined with similar sutures or with wound clamps.

Adult tapeworms were removed from donor rat or hamster hosts. They were pooled in warm 0.85% saline until implantation or until mutilation and implantation. Usually the posterior end of the strobila was transected and discarded and only about 15 to 20 mm of the scolex end was used. Mutilations of scolex and strobila were done free-hand under the stereoscopic microscope using a Bard-Parker No. 11 blade.

At the termination of the experiment the recipient rat was killed and its stomach, small and large intestine were examined under saline. Positions of tapeworm implantation and recovery were noted, the parasite was allowed to flatten naturally on a large glass plate in a small amount of saline, and was then fixed in Gilson's fixative. Tapeworms were stained with Delafield's hematoxylin. Mutilated tapeworms were fixed in Zenker-Formol fixative, were sectioned transversely in paraffin at 5 microns, and were stained in Hansen's trioxyhematein-Masson's trichome (Pantin, 1948) or Heidenham's hematoxylin stains.

RESULTS

Transfaunated *H. diminuta* succeeded in establishing themselves in the small intestine of recipient rat hosts, but died when placed in the large intestine. Eight hosts had a total of 20 tapeworms implanted in the small intestine; 17 tapeworms were recovered at autopsy. Nine tapeworms were placed into the large intestine of 4 hosts; no tapeworms were recovered at autopsy.

Regardless of the site of implantation in the small intestine adult tapeworms succeeded in crawling forward or backward to reach their normal position, with the scolex usually 5 to 30 cm behind the pyloric valve. In a typical case tapeworms were implanted 54 cm behind the pyloric valve (total small intestine length was 61 cm) and at autopsy 8 days later the scoleces were attached 10 cm behind the pyloric valve. During the 8 days they had moved forward 44 cm from the point of implantation.

Growth of transfaunated tapeworms was also rather rapid. After transecting and discarding the posterior end of the strobila, approximately 15 to 20 mm, as mentioned above, of the anterior end was implanted. After 4 days, three implanted worms were 59 mm average length; after 8 days, two were 162 mm average length; and after 14 days, three were 373 mm average length. Ten-day old tapeworms grown from cysticercoids averaged only 113 mm in length which is approximately 27 mm less growth than 8 day implants achieved.

In every case of successful transfaunation the scolex and immature regions at autopsy were normal in appearance. In the 4-day implant the posterior proglottids had progressed only to the mid-mature stage. Sex organs were compact, well-stained and completely differentiated, but without evidence of ovarian function. After 8 days gravid proglottids with many eggs in the typical follicular pattern were evident. The 14-day implants were shedding many ripe eggs with completely-formed, shelled hexacanth embryos. In several instances interesting interruptions in the tightly-packed gravid proglottids occurred; these interrupted proglottids were clear and devoid of sexual products.

Survival and repair of mutilated tapeworms was accomplished in the small intestine. Notches cut in the immature zone quickly disappeared and were not evident after several days. Results of mutilation of the scolex fell into definite patterns. Complete ablation of the scolex resulted in failure to survive; however, some de-scolexed tapeworms have persisted in the gut for about 12 hours. These ex-

hibited crawling motions which appeared to start at the truncated anterior end, which was capable of expansion and contraction as in the normal scolex. After 24 hours de-scolexed tapeworms were absent from the intestine apparently having been carried posteriorly by peristalsis and eliminated. Removal of 1 or 2 suckers (Fig. 1) from the scolex was followed by successful establishment of the worm; on the other hand, removal of 3 suckers led to loss of the tapeworm within a week and perhaps sooner.

Twenty-one rats had implanted a total of 85 tapeworms mutilated by loss of from 1 to 3 suckers. Two rats implanted with 9 tapeworms seemingly devoid of 3 suckers failed to reveal any worms at autopsy; 13 rats implanted with 48 tapeworms devoid of 2 suckers revealed 20 attached and vigorous worms at autopsy. Four rats into which had been implanted 19 tapeworms lacking 1 sucker contained 5 worms at autopsy. Two other rats into which 9 mutilated tapeworms had been implanted revealed at autopsy 1 dead worm from 1 dead host; this tapeworm was not processed. The second host was alive when sacrificed, but revealed no tapeworms. One rat and tapeworm count is mentioned twice in the above totals since 5 mutilated tapeworms had been implanted in 1 host from which 2 tapeworms (1 with 2 suckers, and 1 with 1 sucker missing) were recovered. It was difficult in the mutilation operation to be sure what had been removed since worms were not held outside the host for prolonged observations. The time for removal, mutilation and reimplantation rarely exceeded about 5 minutes. Identification of type of mutilation was made after recovery and staining of all tapeworms.

Anterior and posterior migration from points of implantation occurred in mutilated as in normal tapeworms. This suggests reorganization of nervous control since longitudinal bisection of the scolex to remove 2 suckers certainly removed some nervous elements of the scolex. Yet sucker tenacity was pronounced in the 2-suckered worms and they had to be forcibly removed from the mucosa. The mutilated scolex also made the usual breast-stroke-like motions of the undamaged scolex. Tapeworms with damaged scoleces have been recovered from the gut from 4 hours to 27 days after implantation. The former were near the point of implantation, the latter within 15 to 20 cm of the pyloric valve. Recovery from shock of the mutilation was rather prompt. In one series of experiments, 5 hours after implantation tapeworms were found about 20 cm below the site of implantation, after 12 hours some were already 3 cm anterior to the point of implantation.

Reproductive behavior of mutilated tapeworms is also normal. Ripe eggs were evident in gravid proglottids of worms allowed to remain in the host for sufficient time.

Within 4 hours post-implantation of a mutilated worm the scolex already had initiated repairs (Figs. 2, 3, 4, 5). The wound area was covered with a thin cuticula and was often lifted in a blister-like wheal. The interior of the blister was filled with loosely-organized cells which were iridescent when illuminated by intense light from above in freshly fixed specimens. The undamaged cuticula adjacent to the wound showed folds (Figs. 3, 5, 9) which suggests an elasticity causing it to retreat from the traumatized area. By 7 to 12 hours post-implantation (Figs. 6, 7, 8, 9) little change had occurred over the 4 hour condition. The vesicular wheals were still present and the cavity contained similar cell types. After 1 day (Figs. 10, 11) the scolex, in living organisms, had the appearance illustrated. If the vesicular blisters in Figures 2 to 9 are not the results of hypotonic fixatives then a return

to smooth, decreased contours was established during the first day (Figs. 10, 11). This condition persisted after 2 days (Fig. 12) and subsequently. Figure 12 illustrates an interesting tandem orientation of the remaining 2 suckers; furthermore, this scolex was functionally normal and the worm was firmly adherent to the mucosa.

Histological examination of sectioned scoleces revealed the prompt repair of the cuticula, but no regeneration of suckers. Within 4 hours after implantation the wound was covered with a thin, somewhat chromophobic cuticula seemingly secreted by a subepidermal layer, possibly derived from the parenchyma of the scolex, which moved quickly to the cut surface. This newly formed cuticula was loose and marked by thin processes which ran radially to the surface from large cells located at the base of the cuticula. The new cuticula was thickened, finely granular, and appeared vacuolated in contrast to the compact homogeneous undamaged cuticula on either side. Both new and old cuticula bore fine villous processes (Read, 1955) at the outer margin, but these were larger, longer, and coarser in the new secretion.

Older repaired cuticula, 27 days post-implantation, was still thicker than undamaged cuticula and possessed somewhat shorter and less coarse villous processes, but had lost evidence of most of the cytoplasmic strands and the vacuoles present in earlier repair specimens. In these older implants subcuticular muscle fibers were also evident. Nervous elements were still present, of course, on the undamaged side of the scolex, but were not identified on the damaged side. A more complete analysis of these results will be presented in a subsequent report.

As mentioned above, in even the oldest implants, suckers were never regenerated. Although decreased in number by 1 or 2 the remaining suckers were capable of maintaining a tenacious grasp on the mucosa and were capable of assisting the organism to move forward from points of implantation to zones of optimal enteric conditions as in worms with normal scoleces.

Post-mutilated tapeworms when removed from recipient hosts moved normally when transferred to saline. The scolex was still the obvious center of control and waves of contraction were initiated here. The organisms appeared as well integrated as non-damaged specimens.

The scolex is obviously a chief center of control since de-scolexed worms, as mentioned above, were quickly lost; furthermore, de-scolexed specimens did not maintain themselves in the intestine for any time even when assisted by artificial methods. De-scolexed specimens were tied securely in their immature region with 6-0 sutures and anchored surgically in the most favorable region of the anterior intestine. These specimens, too, were quickly lost and were never recovered at autopsy after 24–48 hours.

DISCUSSION

Surgical implantation of tapeworms is a successful, simple, and convenient tool for use in many types of studies. This method is, in some ways, better for establishing single worm infections than is the feeding of a single cysticercoid; success, or failure, can be more rapidly ascertained by examination for fecal eggs, than for worms grown from cysticercoids. Larger pieces, even to whole tapeworms, may start shedding eggs within a few days.

Mortality of hosts and worms was considered satisfactory in these studies. Only one of 21 rats, and 3 of 20 normal tapeworms used in these experiments failed to

survive until sacrificed. In long-term mutilation experiments, ranging up to 27 days post-implantation, 27 of 85 tapeworms (32%) were present at autopsy. However, short-term experiments (4–12 hours post-implantation), performed recently, have given better recovery rates; 15 worms were recovered from 20 implanted (75%). It is uncertain whether our techniques have improved or whether longer times jeopardize survival.

Tapeworms implanted into the small intestine were generally recovered at autopsy. Furthermore, the ability of implanted adult worms to migrate is similar to the movement, reported by Chandler (1939), of ingested *H. diminuta* which usually excysted 40 cm or more behind the stomach and moved to more anterior stations between the seventh and tenth days.

Worms implanted into the posterior small intestine had lesser recovery rates than from implants into the anterior small intestine. This loss was probably due to their being carried into the colon before recovery from shock. As mentioned above, tapeworms placed in the colon were never recovered at autopsy.

The colon is definitely an unsatisfactory habitat for *H. diminuta*. Whether this is due to lack of suitable nutrients, or to other changes in the physical environment is not known. It is a fact, of course, in contrast to some parasitic protozoans and trematodes, that tapeworms are not found naturally in the colon. It would be interesting to alter, by surgery, the character of food entering the colon to determine whether the nature of food available in the colon is the limiting factor in cestode survival there.

Mutilated worms recovered from shock rather promptly. Often during the mutilation operations zones of opacity developed adjacent to areas of trauma, but even these worms when implanted established themselves in the intestine. The unexpected feature in mutilated worms was the rapid repair of the traumatized zone. Within a few hours the wound of the scolex was covered with a new cuticula in which was embedded cellular debris including fibers of the subcuticular muscles. As mentioned above, this new secretion offers opportunities to study the structure and chemical nature of the cuticula since it can be started and stopped at will. Presently we have experiments under way to examine the new cuticula chemically and with the phase and electron microscopes. Smyth (1948) studied behavior of fragments of plerocercoid larvae of *Ligula intestinolis in vitro*. He reported decreased activity and failure of tissue regeneration; however, fragments did produce eggs in a manner similar to whole plerocercoids.

The outer surface of the repair cuticula from the beginning had a villous-like appearance. These processes were coarser, in comparison to cuticular thickness, than those reported by Read (1955) in normal cuticulas. Later they appeared shorter and less coarse; whether this indicates a sloughing of the first, and a production later of finer ones is as yet unknown. In some sections, 5 microns thick, which were further squashed to reduce thickness, fine parallel striae, perpendicular to the surface, were apparent in the cuticula. The real or artifactitious nature of these will be determined in subsequent studies.

SUMMARY

Adult rat tapeworms, *Hymenolepis diminuta*, were successfully implanted by surgical methods into the small intestine of recipient Wistar white rats. Seventeen of 20 worms implanted into 4 hosts were recovered after varying periods of time.

Worms succeeded in crawling from implantation sites into regions of optimal character. Growth recovery was rapid: anterior pieces 15 to 20 mm long at implantation were 59 mm long after 4 days, 161 mm long after 8 days, and 373 mm long after 14 days. Reproductive structure and behavior in implants were also normal; egg production had commenced by 8 days, and viable onchospheres appeared in the feces after 14 days.

Repair of mutilated scoleces and strobilae was also prompt. Complete ablation of the scolex resulted in inability of the worm to survive. Removal of 3 suckers resulted also in loss of worms. Worms survived with 1 or 2 suckers removed. Seventeen rats which received 67 worms lacking 1 or 2 suckers revealed, at autopsy, 25 vigorous and firmly attached worms. These worms also migrated and developed normal strobilar morphology in the small intestine. Cuticular repair was rapid. Within 4 hours a new, loose and vacuolated cuticula was deposited on the wound area. A gradual return to near-normal histology of the cuticula occurred during the next 27 days. Excised suckers were not regenerated.

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Explanation of Figures

All figures were drawn free-hand, and only roughly to the same scale.

Figure 1. Diagram of intact scolex. The dotted line indicates the lines of incision to remove 2 suckers.

Figures 2 and 3. Two views of the same fixed scolex, 4 hours post-implantation (Susa's fixative).

Figures 4 and 5. Two views of the same fixed scolex, 4 hours post-implantation (Bouin's fixative).

Figures 6 and 7. Two views of the same fixed scolex, 7 hours post-implantation (Susa's fixative).

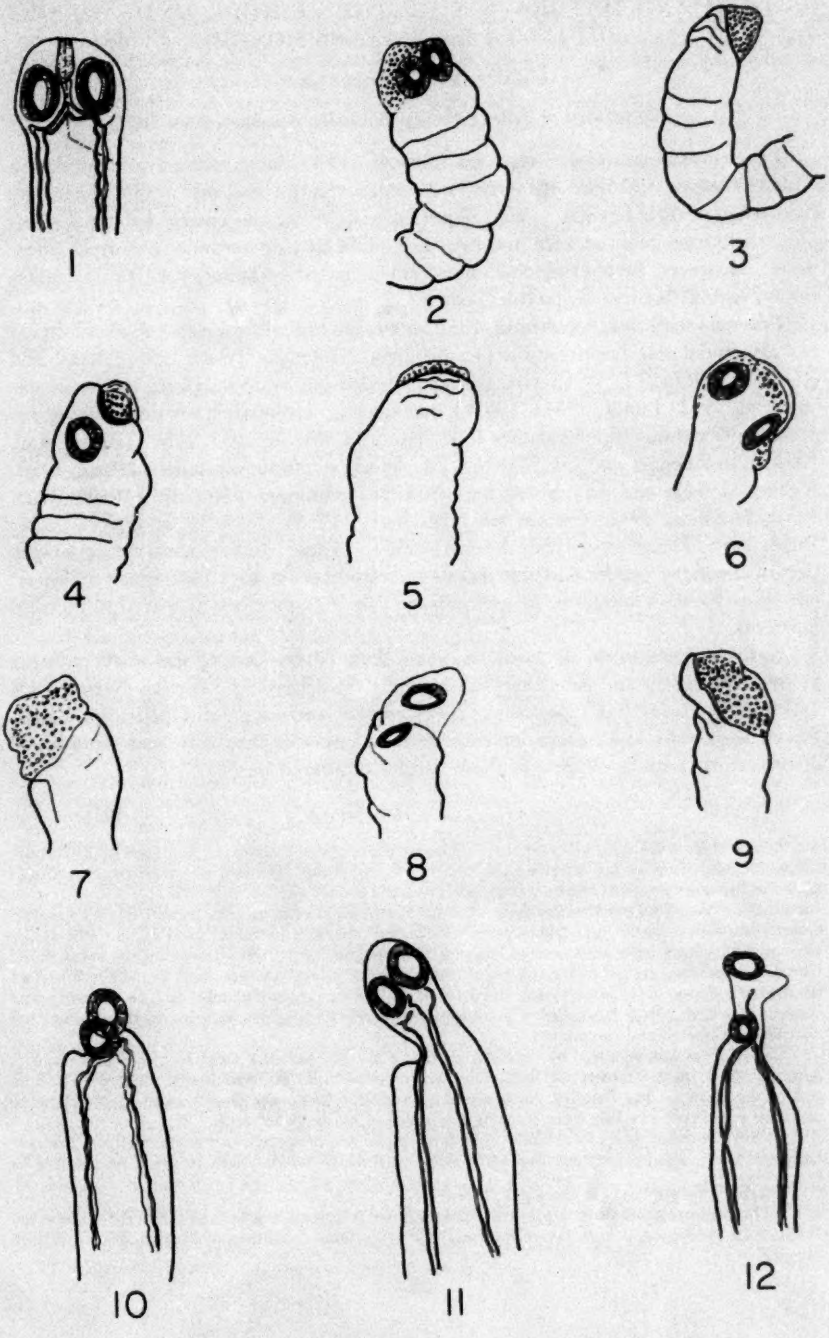
Figures 8 and 9. Two views of the same fixed scolex, 7 hours post-implantation (Bouin's fixative).

Figure 10. Living scolex, 1 day post-implantation.

Figure 11. Living scolex, 1 day post-implantation.

Figure 12. Living scolex, 2 days post-implantation.

PLATE I



GROWTH AND MATURATION OF THE CESTODE *HYMENOLEPIS DIMINUTA* IN BILELESS HOSTS*

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Tapeworms, with few exceptions (Smyth, 1954), have resisted attempts at in vitro cultivation. Failure of in vitro maintenance is due, in part, to our incomplete knowledge of their normal in vivo requirements. Since the enteric habitat is complex, their total requirements are extremely difficult to determine in normal infections. However, by altering this environment, factor-by-factor, relative dependencies on normal factors may be fractionated.

Previous work (cf. von Brand, 1952 for review of literature prior to about 1950) has attempted this fractionation (1) by dietary methods (Beck, 1951; Read and Rothman, 1957), (2) by altering the host's endocrine balance (Beck, 1952; Chandler, et al, 1952; Landt, 1954), (3) by introducing, withholding, or determining the presence of various supplementary food factors (Coleman, 1953, 1956; Dacres, et al, 1953; Kaipainen, et al, 1954a, 1954b), (4) by use of tracer substances (Read, 1950; Nyberg, 1953), and (5) by various in vitro techniques (Hopkins, 1950; Read, 1951; Hopkins, 1952; Garson and Daugherty, 1953; Goldberg and Nolf, 1954; Read, 1956; Daugherty, 1957; Laurie, 1957). These studies, constituting only a part of the many papers on these phases of cestode physiology have given a clearer, but by no means a complete, insight into the role of luminal as compared to mucosal nutrients.

In the present work, rat hosts have had their biliary flow to the small intestine altered by surgery and have been subsequently challenged by infective cysticercoids or implanted adult rat tapeworms, *Hymenolepis diminuta* (cf. Goodchild, 1958). Survival, growth, and maturation rates of these cestodes have then been determined after varying periods of time in these surgically altered hosts.

MATERIALS AND METHODS

Wistar rats were used as donor and recipient hosts and the confused flour beetle, *Tribolium confusum*, was used as the intermediate host of *H. diminuta*. Hosts and parasites were maintained in the laboratory according to methods given previously (Goodchild, 1958).

Surgery was performed under light ether or nembutal anesthesia; the former gave somewhat better results with more rapid post-operative recovery and was therefore used in the bulk of the experiments. Rats were anesthetized, clipped, and swabbed with 70% ethanol on the right flank. The duodenum was pulled to the outside through a small lateral incision, and the whole bile duct uncovered. Since only the anterior third of the bile duct, approximately, is free of pancreatic ducts it was tied with 6–0 braided silk sutures at this level. Slightly anterior to the ligation the duct was transected and cannulated.

Cannulation was achieved by carefully inserting into the lumen a short length of polyethylene tubing (0.023" inside diameter, 0.038" o.d.) which was tied into place with silk sutures. The free end of the cannula was handled variously in several different experiments most of which were designed to prevent free bile from entering the lumen of the small intestine.

1. *Bile draining freely to outside.* The free end of the cannula was run through the primary incision, or through a secondary one made more posteriorly, and allowed to drain on the side of

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the body. This of course permitted the rat to lick bile from the flank and hence results may not be conclusive.

2. *Bile draining into protected latex bags (finger cots) anchored to the flank or back.* The cannula was run through a cork on which was placed a finger cot protected by a plastic cylinder. Each day the bile was withdrawn from the finger cot by means of a hypodermic needle and syringe introduced through the cork.

3. *Bile draining into the vas deferens.* The cannula was anchored into the right vas deferens after the rat had been unilaterally orchectomized. Bile drained into the urinary tract and was voided with the urine.

4. *Bile draining into the cecum.* The cannula was stitched into the apex of the cecum and the bile introduced directly into the large intestine. Since this operation seemed most promising in total elimination of bile from the small intestine several subseries were run in which supplementary bile, bile salts, and inorganic alkalizers were added to food eaten by the cannulated hosts.

A. *Bile to cecum and the food fortified with dried ox bile.* Dried ox bile was mixed in the proportions of 0.5%, 1%, or 2% ox bile in pulverized Laboratory Chow.[1]

B. *Bile to cecum and the food fortified with sodium glycocholate.*[2] The latter was administered in the proportions of 0.5% or 1% in pulverized Chow.

C. *Bile to cecum and the food fortified with sodium taurocholate.*[2] The latter was given in the proportions of 0.5% or 1% in pulverized Chow.

D. *Bile to cecum and the food fortified with an alkalizer* consisting of 3% magnesium trisilicate and 3% calcium carbonate in pulverized Chow. The animals were given water to which was added 0.5% $NaHCO_3$.

5. *Double ligation and transection of the bile duct.* Transection was performed above the zone of confluence with the accessory pancreatic ducts. In this series bile was prevented from entering the small intestine and was allowed to accumulate in the biliary passages.

6. *Control rats were of two sorts.* In the first, the bile duct was cannulated as above, but the free end of the cannula was returned to the small intestine at the usual site; in the second, a sham operation was performed with similar degrees of trauma to the tissues, but without cannulation.

Food was withheld from post-operative rats for usually 24–48 hours. Water was usually provided from the time of operation on. When food was offered to rats immediately after their operation, it was observed that none was eaten for about 24 hours. Labratory Chow and the special diets were provided ad libidum to all experimental hosts.

The scolex, together with approximately 50–80 mm of anterior strobila (henceforth referred to as anterior fragments), cut from larger tapeworms recovered from donor hosts were administered to surgically altered rats at the time of initial operations or after recovery. Usually these anterior fragments were placed in the intestinal lumen at the time of the initial operation; in a few cases they were implanted within 4–5 days after the rat had nearly recovered from the effects of the previous operation. Cysticercoids were generally administered orally after recovery of the host. No differences were observed as a result of the time of implantation and, therefore, the following account includes both types. Parasites were allowed to remain in the host for varying periods of time before sacrifice.

Rats were sacrificed, the small intestine, large intestine, and stomach removed and examined under saline. Sketches and photographs of the host organs and parasitic burden were made at the time of post-mortem examination. The complete digestive tract was pinned on wax under saline and examined with the stereoscopic microscope. Tapeworms were recovered, were allowed to flatten in saline on large glass plates, and were fixed in Gilson's fixative. All tapeworms were stained as whole mounts in Delafield's hematoxylin.

RESULTS

Seventy-eight rats were used in all of these experiments; of these, 44 (56%) lived until sacrificed. Those which died survived an average of 6.1 days; some of these were already decomposed when found dead and were not examined for parasites. Others were examined immediately after death and the presence or absence of tapeworms determined. Since most of these operations involved delicate and serious surgery the overall survival rate was considered satisfactory. Some operations had higher mortality rates than others, e.g., 6 of 16 rats survived cannulation of the bile duct to the outside or into latex bags, 2 of 9 survived cannulation of the

[1] Ralston Purina Company, General Offices, St. Louis, Missouri.

[2] Nutritional Biochemicals Corporation, Cleveland, Ohio.

bile duct to the vas deferens, 19 of 27 survived cannulation to the cecum, 9 of 15 survived bile duct ligation and transection, 6 of 9 survived cannulation of the bile duct back to the duodenum, and 2 of 2 survived the sham operation.

In the subsequent results only the animals living until sacrificed or examined immediately after natural death will be discussed. These will be categorized under the headings as given in the previous section.

1. *Bile draining freely to outside.* Five rats were fed a total of 47 infective cysticercoids; animals sacrificed or autopsied after an average of 6 days post-ingestion failed to yield any young tapeworms.

2. *Bile into latex bags.* Approximately 10–15 ml of bile were produced daily by each cannulated rat. Only slight weight losses were noted in these hosts (approximately 1 gram per day). For example, rat #39 decreased in weight from 362 grams to 352 grams in a 13-day experimental period; rat #43 decreased from 380 grams to 375 grams in 5 days. Rats living until sacrificed were generally in excellent condition and the inner end of the cannula was anchored firmly in place. There was no evidence of leakage of bile into the coelom.

From 3 rats into which 9 tapeworm fragments approximately 50–80 mm long had been surgically implanted (Goodchild, 1958), 5 tapeworms were recovered at autopsy. These had remained in their hosts for 5, 8, and 13 days post-implantation, and averaged only 14.1 mm in length when recovered. Growth had not occurred in any host, and in 1 host (8 days post-implantation) the tapeworm had decreased to 1.6 mm in length. The general morphological condition was also atypical in comparison to tapeworms of similar size implanted into normal hosts (Goodchild, 1958). Fragmentation and increased opacity were observed; eggs were never observed even in the 13-day implants whereas they were invariably present in 13-day implants in normal hosts (Goodchild, 1958).

3. *Bile to vas deferens.* One rat had implanted 3 anterior fragments of *H. diminuta,* and 2 rats were fed a total of 21 infective cysticercoids. These rats were autopsied 10, 7, and 19 days post-implantation or ingestion and revealed no tapeworms from the implants, and only 2 (from 7-day hosts), averaging 8.1 mm in length, from the cysticercoids. These worms were stunted and atypical. The cuticula was thicker and looser than normal and there was no evidence of reproductive Anlagen. The scolex of one of these worms was shorter than usual and the immature zone was devoid of incipient proglottids.

4. *Bile to cecum.* As mentioned above, this series appeared to be most promising in determining biliary effects upon adult tapeworms and was accordingly investigated more thoroughly. Some weight losses of hosts were noted. Seven sacrificed, or recently dead, rats had an average weight of 325 grams initially, and had average weights of 311 grams at the end of an average 8.4-day experimental period; this represents an average daily loss of 1.7 grams per host. Furthermore the weight losses were nearly uniform in each host. The nutritional condition of the hosts was generally good. The fur was sleek and smooth, and the internal organs were normal, with food in the gut.

Six tapeworms were implanted into 2 hosts and 10 cysticercoids fed to 1 host. At autopsy 3 tapeworms were recovered from implants, but none from cysticercoids. The implants had an average length of 55 mm. This may be a slight increase in size, but was much less than the 373-mm length achieved by 14-day im-

plants in normal hosts (Goodchild, 1958). The tapeworms, furthermore, were nearly transparent, thin, and weak.

The recovered implants also showed abnormalities in morphology and functional activities. Although the scolex and immature region appeared normal they were imbedded in strands of mucosa. The anterior end of the worm was frequently buried in the mucosa which clung to the scolex in strands even after careful removal performed under the stereoscopic microscope. Sex organ Anlagen developed in posterior immature proglottids, but these failed to differentiate normally in the mature zone. Seminal vesicle rudiments were slightly differentiated, but testicular masses were pale, diffuse and practically nonfunctional. Only occasional receptacles contained sperm. There was no evidence of vitelline or ovarian function. This again is in sharp contrast to functional maturity of implants, in equivalent time, in normal hosts.

A. *Bile to cecum and the food fortified with dried ox bile.* A total of 7 rats was used in this series of which only 2 died, after 4 and 5 days. The remaining ones lived an average of 11.2 days before sacrifice. Weight losses again were recorded. The 5 rats weighed initially an average of 316 grams, and finally an average of 266 grams. During the 11.2 days they lost uniformly an average of 4.5 grams daily.

In this series, tapeworm growth from implants and cysticercoids was better than in any previous series. Two rats, fed 1.0% ox bile, received only implanted tapeworms. From one of these 4 worms were recovered, after 8 days, from 4 implants; from the second rat no worms were recovered, after 9 days, from 2 implants. From two other rats, fed 0.5% and 1.0% supplementary ox bile and a total of 15 cysticercoids, 3 worms were recovered. The fifth rat, fed 2% ox bile, received 3 implanted worms and 10 cysticercoids orally; 9 worms, 3 large and 6 small, were recovered after 10 days. These were easily classified as to origin from implants or cysticercoids.

The size of recovered worms was roughly proportional to amounts of supplementary bile in the basic food. The single rat receiving 2% ox bile yielded large worms averaging 183 mm in length, from implants; and smaller ones averaging 17 mm in length from larvae. The rat receiving 1% ox bile yielded, from implants, worms averaging 60 mm in length. Similar size increments were observed in worms developed from cysticercoids. Ten cysticercoids fed to the host supplemented with 2% ox bile yielded 6 worms averaging 17 mm after 10 days; this was the same host from which 3 worms averaging 183 mm were recovered; 5 cysticercoids yielded 2 worms averaging 108 mm in hosts supplemented with 1% ox bile. The stunting here with increased bile supplement, may reflect the crowding effect reported by Chandler (1939), cf., also review by Read (1951). Unfortunately the single worm recovered from a rat supplemented with 0.5% ox bile was lost in preparation; its measurements, obviously, are not given.

In addition to support of growth, supplementary bile appeared also to encourage normal maturation and function. Most tapeworms in this series were completely typical with well differentiated and functional sex organs. In the mature zone the testes, ovaries and accessory organs were compact, deeply stained, and revealed intense meiotic activity in the primary organs. Many ripe eggs were visible in the gravid proglottids.

B. *Bile to cecum and the food fortified with sodium glycocholate.* In preliminary

experiments to ascertain roles of bile salts, pulverized Laboratory Chow was fortified with sodium glycocholate. Three rats were implanted with 9 tapeworms of which 3 were recovered from 2 hosts after 10 days. One of these successful hosts, fed with 0.5% sodium glycocholate, gained 6 grams in weight in 10 days; the other, also fed 0.5% glycocholate, from which 1 tiny worm (1.8 mm) was recovered, lost 60 grams in the same time. This last host was sick and weak, and revealed, at autopsy, a ruptured bile duct with copious bile in the coelom. The other host revealed 2 worms averaging 38 mm in length. The worms were normal in morphology and function. Testes were large, distinct, functional, but somewhat vesiculated. The ovary was also discrete, well stained, but, at 10 days, had not commenced egg-output.

C. *Bile to cecum and the food fortified with sodium taurocholate.* Three rats, sacrificed after 7, 9, and 10 days, had implanted 10 tapeworms from which 6 worms were recovered from 2 hosts fed 0.5% and 1% supplement. The third host, receiving 0.5% taurocholate, revealed no worms. Again weight was lost. Initially weights were an average of 257 grams, terminally they were 237 grams. Over an average period of 8.7 days each rat lost 2.3 grams daily.

Implanted worms averaged approximately 50 mm in length and recovered worms, after 8.7 days, averaged 47.9 mm in length. They barely maintained original size during the experimental period. Worms from the two hosts were all nearly the same size.

Recovered tapeworms showed rather normal morphology and function. Scoleces and immature zones were typical; mature zones revealed compact, deeply stained, and functional testes. Seminal receptacles were packed with sperm. Some worms showed normal ovarian function, others delayed function. In the former, uterine areas contained eggs not yet advanced to onchospheres; in the latter, the ovaries and yolk glands were filled with a homogeneous granular mass without evidence of incipient egg-formation. It was considered possible that with additional time ripe, infective eggs would have been shed by some of these parasites.

D. *Bile to cecum, food and water supplemented with alkalizers.* It was considered possible that effects previously noted might be influenced also by altering the alkalinity of the small intestine. Two rats had implanted 6 tapeworms, and 1 rat was fed 10 infective cysticercoids; after 7, 9, and 13 days, hosts were sacrificed, but tapeworms were totally absent. Rats were in excellent condition with cannulas in place and normal quantities of digesting food in the intestinal tract. Weight losses occurred, however, in spite of ample ingestion of food. The 3 rats weighed an average of 260 grams at the start and 243 grams terminally. Over an average experimental period of 9.6 days each rat lost 1.7 grams daily.

5. *Bile duct ligated and transected.* Three of the 15 rats used in this series were operated, but not challenged with tapeworms. Weight losses were observed in these 3 and in 1 rat of the experimental series. From an initial average weight of 260 grams 4 rats dropped to average weights of 237 grams in an average of 11 days; this decline represents a 2.1-gram daily loss for each host.

All rats developed jaundice with extremities, skin, muscles and viscera becoming increasingly yellowish in color; yet most appeared to remain in satisfactory over-all condition. Most ate well and the gut, at autopsy, contained partially digested food. In 1 rat the lumen of the gut contained copious amounts of mucus and the mucosa

appeared to be hyperemic and to be sloughing in a few patches. From this one host 3 of the largest tapeworms, from 3 implants, were recovered at autopsy; these worms ranged from 73 mm to 122 mm (average 98 mm) in length.

Three hosts were fed 10 cysticercoids each; after 12.3 days average infection time, no young tapeworms were recovered at autopsy.

Eight other hosts had implanted 23 *H. diminuta* anterior fragments each 50–80 mm long. From these hosts 13 tapeworms were recovered. These worms ranged in average size from 3 to 98 mm. The overall average of the 13 worms was 46.2 mm from average post-implantation times of 16.7 days. Although length had increased in some worms it fell far short of length of tapeworms (average 403 mm) implanted for equivalent times in normal hosts.

Morphologically and functionally these worms were abnormal. In the smallest ones proglottid-formation was absent and the worms, except for the scoleces, ap-

TABLE I. *Results of tapeworm growth in cannulated hosts with bile returned to the duodenum.*

Rat number	Days post-implantation	Weight change in grams	Tapeworms (t) implanted or cysticercoids (c) ingested	Number (and length, in mm) of tapeworms recovered	Condition of host
45	6	..	4 t	3 (14.9, 21.4, 20.0)	diarrhetic, massive infection, bloated, no food in gut.
49	7	210—220	3 t	0	little infection, tubing in place and draining.
55	10	230—230	10 c	9 (42.4, 42.8, 56.9, 34.9, 43.4, 29.7, 24.6, 25.8, 28.7)	good, food present in intestine.
57	13	220—205	10 c	10 (19.2, 12.1, 7.3, 13.5, 10.5) (5 only stained)	bile duct blocked and distended.
81	11	..	3 t	3 (108.6, 86.0) (2 only stained)	excellent but little food in gut.
99	21	232—253	5 c	1 (191.9)	excellent, gut full of food.

peared plerocercoid-like. In larger ones the sex organs were abnormal with pale, diffuse, non-functional masses of cells in the testicular, ovarian and vitelline zones. In the largest worms some few receptacles contained scanty sperm, and ovarian function had commenced, but the uterine pouches were filled with amorphous cell masses which were considered egg-breakdown products. Normal young or mature oncospheres were never present in any worm.

Internally, as mentioned above, the host viscera were yellowish in color and the ligated bile duct was greatly distended. In some it approached in size the dimensions of the cecum and the large intestine, and stretched from the liver to the urinary bladder. The distention of the duct reached a maximum length of 90 mm and 20-mm width in a 22-day post-ligated host. This bile duct contained 1.3 ml of bile. Since rats secrete 10–15 ml of bile daily, 220–330 ml of bile may have been produced but not discharged into the gut in this time.

6. *Control series: Bile duct cannulated and re-routed to duodenum.* As mentioned above, 6 of 9 rats survived this type of cannulation. In some the operation was completely successful and weight gains were recorded; in others, blockage of the cannula occurred with resultant bile duct distention and the usual weight losses noted above. Growth and maturation of tapeworms were directly correlated with

the overall success of biliary return to the small intestine. Since these data are crucial, detailed information is presented in Table I.

Only 1 host (#49) failed to yield worms from implants. From 10 implants (approximately 15–20 mm long) in 2 other hosts 6 tapeworms were recovered. Six-day implants had retained the size of implants, and the mature regions showed reproductive organs. Spermatogenesis, however, was not pronounced and ovaries had not commenced egg-production. Eleven-day implants had increased greatly in size and the worms were normal in appearance and function. These worms were as large as implants into normal hosts and gravid proglottids were packed with eggs in the process of shell-formation. Worms, 10 days old, from cysticercoids in host #55 overlapped sizes of 10-day worms reported by Chandler (1939). These worms revealed normal sex organs with testes functioning. In some, ovarian and vitelline activity had commenced, although gravid proglottids were not yet present. With additional time it was considered inevitable that normal oncospheres would have been shed from host #55. The worm from host #99 was shorter than worms from implants into normal hosts, but did reveal normal sex organs and gravid proglottids. The latter were packed with eggs in which larvae with differentiated hooks occurred. Salt flotation of intestinal contents revealed a few normal oncospheres.

7. Control series: sham operation. Only 2 hosts, which lived until sacrificed 13 and 15 days post-operatively, were used. These hosts gained in weight an average of 0.7 grams daily. Initial average weights were 250 grams and final weights, after an average of 14 days, were 260 grams.

Three tapeworm fragments were implanted into each rat. At autopsy 3 were recovered from each. Thirteen-day infections yielded completely normal and functioning worms averaging 406 mm in length; 15-day infections also revealed normal functioning parasites averaging 345 mm in length. This series was not extended since it appeared to duplicate succesful work previously reported (Goodchild, 1958).

DISCUSSION

Lack of bile appears to exert profound effects upon adult tapeworms. Growth in bileless hosts was markedly depressed as was sexual maturation. From all hosts deprived of bile 8 (44.5%) of 18 implanted worms were recovered, and only 2 worms (2.6%) from 78 ingested cysticercoids. Comparison of total lengths of implanted fragments (average 50–80 mm) to total lengths of recovered worms (average 34.5 mm) is striking. From 900 to 1440 mm (18×50–80 mm) of implants only 276 mm (8×34.5 mm) of tapeworms were recovered after an average infection period of 11.2 days. This compares unfavorably with recovery percentage and lengths achieved by implants into normal hosts (Goodchild, 1958). Seventeen worms (85%) in this normal series were recovered from 20 implants. Since growth increases fell nearly on a straight line, based on data calculated from the earlier paper, at 11.2 days these worms, by interpolation, average 265 mm in length. Thus from 300 to 400 mm (20×15–20 mm) of implants 4505 mm (17×265 mm) of tapeworms were recovered. Worms recovered from cannulated hosts averaged approximately 23% (276 mm: 1170 mm) the size of implants, whereas in normal hosts they averaged nearly 1300% (4505 mm: 350 mm) the size of implants.

In bileless hosts excystment and growth of cysticercoids was also subnormal.

Two recoveries from 78 ingested larvae seem to be much below usual limits; for example, Chandler (1939) reported 90% yield of worms from as many as 40 cysticercoids per host. Moreover, in only 1 series, where cannulation of the bile duct to the vas deferens was performed, were tapeworms found. The average lengths, 8 mm, achieved by these 2 worms in 7 days falls below limits of normal growth (average 23 mm) in similar times reported by Chandler (1939). As mentioned earlier, self-cleansing of the groin may have introduced some bile into the digestive tract to account for these.

The small daily weight losses in host rats was not considered the limiting factor in growth and maturation of the adult of *H. diminuta*. In an unpublished experiment host rats were maintained for varying periods of time on reduced food allotments. From these hosts, some of which lost weight at equivalent or greater rates than in the current series, apparently normal worms were recovered. For example, in a series given daily only 9 grams of Laboratory Chow for 14 days (a rat may consume nearly 15 grams of Chow each day), each rat lost an average of 14 g, or 1 g daily. Yet from these rats normal-appearing tapeworms averaging 260 mm in length were recovered from implants. Although approximately 100 mm shorter than tapeworms from hosts fed to repletion, they were about 200 mm longer, on the average, than implants from bileless hosts.

Maturation and onset of sexual reproduction were also depressed in bileless hosts. In some implanted worms, from such hosts, proglottids failed to occur even though some growth in length occurred. In most, the rudiments of the sex organs in the immature zone were pale and diffuse. If sex organs were present only the testes appeared to function, but with greatly reduced sperm output. Receptacles were only irregularly and scantily filled with sperm. The ovary and yolk gland continued as scattered cell masses without sharp, clear boundaries in stained specimens. After an average of 8 days post-implantation as reported in the previous paper, Goodchild (1958), tapeworms in normal hosts were packed with ripe eggs in gravid proglottids. Gravid proglottids were not present after an average of 11.2 days in the present series. The 2 worms from cysticercoids were even more atypical. Although the scoleces were normal and in 1 worm the immature zones had pale central reproductive Anlagen visible, the cuticula was thicker and looser than normal. The same tapeworm also had patches of black material, not entrapped air or xylene vapors, in the parenchyma. The second tapeworm, 2.2 mm long, lacked proglottids after 7 days and there were no reproductive Anlagen.

Cannulated rats with bile entering the cecum and the food fortified with dried ox bile, sodium glycocholate, and sodium taurocholate yielded worms generally of larger size and more normal function than unsupplemented hosts. This increase occurred in spite of the fact that some hosts lost weight at the greatest daily rate. Food to which 1% ox bile had been added yielded 240 mm total length of worms, after 8.5 days, from 300 to 480 mm of implants; 2% ox bile supplement yielded 549 mm total length of tapeworms from 150 to 240 mm of implants in 10 days. Sodium glycocholate added to food in 0.5% concentration produced about 77 mm of worms in 10 days from 250–400 mm of implants; whereas sodium taurocholate in 0.5% supplement supported 93 mm total length from 300–400 mm of implants. Taurocholate in 1% concentration yielded, after 7 days, 4 worms from 4 implants; total length at recovery was approximately 200 mm length from about 200–320 mm of

implants. These data suggest that, of concentrations used, 2% ox bile gave best yields, with 1% taurocholate next best. A most interesting finding in 1 host in the 1% ox bile series was the occurrence of the main portion of the strobila in the rat's stomach. This unusual position has never been noted in normal hosts.

Normal morphology and function of tapeworms was regularly noted in cannulated and bile-supplemented hosts. Testes, ovaries, and yolk glands were compact, deeply stained, and intensely meiotic. Ripe eggs and oncospheres packed gravid proglottids of tapeworms allowed to remain implanted for sufficient lengths of time. Beck (1951) also reported a stimulatory effect of dog bile upon egg output in rat tapeworms from hosts maintained on deficient diets.

Although the addition of alkalizers in food and water achieved elevation of intestinal alkalinity as does bile, tapeworms were not recovered from implants or cysticercoids. Stomach contents, in these hosts, measured between pH 3.7–5.5, duodenal about pH 6, jejunal between pH 6–7, and ileal between pH 7.4–7.7. These values approximate ranges of normal guts from which vigorous tapeworms have been recovered. It seems probable, therefore, that acidity or alkalinity alone are not controlling factors in success or failure of tapeworms in altered hosts. Rather there must be specific nutritional or physical factors in bile which support normal growth.

The exact role of bile in normal growth and maturation of tapeworms awaits additional work, of course. Read (1955) has given an excellent review on the role of bile salts in excystation of larval tapeworms; this will not be discussed further here. Certainly in the rat tapeworm, whole bile seems more growth-supporting than either glycocholate or taurocholate in equivalent concentration. Further work may reveal more effective concentration of the bile salts than manifest in the present study. Although optimal alkalinity itself seems ineffective in supporting normal development perhaps combinations of alkalizers with surface tension depressants, in addition to bile, would be efficacious. It seems highly desirable, too, to test growth of other tapeworms and helminths from other phyla in bileless hosts. For example, in as yet unpublished work, we have ascertained that *Hymenolepis nana* thrives better than *H. diminuta* in the absence of bile.

Surgical occlusion of the bile ducts obviously resulted in biliary congestion and distention. However, the amount of bile recovered from the duct never accounted for the total amount possibly secreted by the liver. Furthermore, in a few hosts some growth had occurred in implants, although no worms were recovered from cysticercoids. This growth may have been supported by secretion of biliary components by the small intestinal mucosa. That this possibility exists in fact has not been experimentally demonstrated in this laboratory, yet there was widespread vascular distribution of bile and in some hosts granular aggregates of yellowish material, presumably derived from bile, occurred in mesenteries and peritoneum. If some of this material were actively secreted into the intestinal lumen the slight growth noted may have been realized. Garson and Daugherty (1953) used the technique of bile duct ligation to ascertain the origin of taurine in tapeworms. On the basis of this work these authors concluded that taurine in worms does not originate from biliary constituents; rather it results from degradation of methionine. Wykoff and Lepeš (1957) used bile duct ligation and cannulation in rabbits subsequently challenged by metacercariae of *Clonorchis sinensis*. They reported direct penetration of the gut by juvenile flukes and recovery of young adults from livers of hosts lacking direct biliary connection to the duodenum.

SUMMARY

Surgical alteration of Wistar rats, subsequently challenged by infective cysticercoids or implanted adult rat tapeworms, *Hymenolepis diminuta*, was used to study the role of bile in the growth and maturation of the adult tapeworm.

Seventy-eight rats, of which 44 lived until sacrificed, were used in the following experiments:—(1) *Bile duct cannulated and draining freely to the outside:* From 47 infective cysticercoids no tapeworms were recovered from these bileless hosts. (2) *Bile draining into latex bags:* Nine anterior pieces, including scolex, of adult tapeworms were implanted; 5 worms were recovered at autopsy, but their average size had greatly decreased from implant size and the morphology and function was atypical. (3) *Bile to vas deferens:* 3 anterior pieces were implanted, but yielded no worms at autopsy. Twenty-one cysticercoids were fed to 2 hosts from which only 2 tiny, atypical, sexually non-functioning worms were recovered. (4) *Bile to cecum:* Six tapeworms were fed to 2 hosts from which 3 worms were recovered at autopsy. These, too, had not achieved much, if any, growth. Morphologically and functionally they were atypical. (5) *Bile duct ligated and transected:* Tapeworms were not recovered from ingested cysticercoids. From 23 implants 13 worms were recovered at autopsy. Growth had occurred in some, but not in others. Morphologically and functionally all were atypical. Some had failed to strobilate, and oncospheres were not produced in any after nearly 17 days.

Control experiments were performed in bile-to-cecum hosts whose diets were fortified with various supplements:—(1) *Dried ox bile supplement:* Growth from implants was roughly proportional to the amount of ox bile fed. Some implanted worms had nearly quadrupled in size in approximately 11 days. Morphologically and functionally these worms were normal. Percentage recoveries and growth of worms from ingested cysticercoids was also nearly normal as was their morphology and function. The major portion of 1 tapeworm, in this series, was recovered from the stomach. (2) *Sodium glycocholate supplement:* Three worms were recovered from 9 implants. Although growth had apparently not occurred, morphology and function were normal. (3) *Sodium taurocholate supplement:* Six worms were recovered from 10 tapeworms implanted into 3 rats. Recovered worms were implant size after 9 days, but were normal with respect to morphology and function. (4) *Alkalizer supplement:* From implanted adults and ingested cysticercoids no tapeworms were recovered at autopsy.

Weight losses occurred in most hosts (approximately 1–2 grams daily), but there was no correlation between weight losses and size or appearance of recovered worms.

It is concluded that bile contains a factor, or factors, apparently necessary for normal growth and maturation of adult *Hymenolepis diminuta* in the rat host.

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THE GLYCOLYTIC PATHWAY IN *TRICHINELLA SPIRALIS* LARVAE*

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Previous studies on the intermediary metabolism of *Trichinella spiralis* larvae have indicated that the conventional tricarboxylic acid cycle and cytochrome system are present in the tissues of this parasite (Goldberg, 1957a). To elucidate further the possible pathways of substrate-utilization by this organism, enzyme studies on the primary steps of carbohydrate-degradation have been carried out. The present communication is concerned with glycolysis in *T. spiralis larvae*. A preliminary report of these results has been presented elsewhere (Goldberg, 1957b).

MATERIALS AND METHODS

T. spiralis larvae were obtained from infected laboratory mice by the acid-pepsin digestion procedure and washed free of contaminants as previously described (Goldberg, 1957a). Homogenates of the parasites were prepared by grinding them plus an equal volume of 0.1M potassium phosphate buffer at pH 7.4 in a small, power-driven, all-glass homogenizer cooled in an ice-bath. For spectrophotometric assays of enzyme activity the homogenate was centrifuged at $12{,}000 \times g$ for 10 minutes at 4°C. In all other experiments the crude homogenate was used.

TESTS FOR ENZYME ACTIVITY:

1. Glycolysis was measured anaerobically in Warburg vessels using glucose as substrate. The reaction system used was modified from LePage (1948). All components of the system were in the main compartment of the flasks except hexose diphosphate (HDP) which was added from the sidearm. The flasks were gassed for 10 minutes with specially purified (99.99%) nitrogen while shaking in the constant temperature bath at 37°C, after which, at zero time, the HDP was tipped in. The control flasks were identical except that 3N HCl, to stop the reaction and determine bound CO_2, was tipped at zero time. Glucose-utilization and lactic acid-production were used as criteria of activity, and were determined in $Ba(OH)_2$-$ZnSO_4$ filtrates.

2. Hexokinase activity was determined by measuring the disappearance of glucose during incubation of a brei fortified with Mg and ATP in an open 25-ml Erlenmeyer flask. The procedure was modified from Boyland et al (1951). After a 5-minute pre-incubation period, the reaction was started by the addition of ATP to the contents of the flask. Aliquots were removed at zero time and at 30 minutes. The net difference in free glucose over the controls was measured according to the enzymatic glucostat micromethod (Keilin and Hartree, 1948).

3. Phosphofructokinase, aldolase, α-glycerophosphate dehydrogenase, glyceraldehyde-3-phosphate dehydrogenase, and lactic dehydrogenase activity were measured spectrophotometrically. The method (in Colowick and Kaplan, 1955) exploits the absorption by DPNH in the near ultraviolet and permits measurement of one or several coupled reactions when DPN or DPNH is required as a coenzyme. Readings of optical densities were made every 15, 30, or 60 seconds using the Beckman Model DU spectrophotometer. Blank cuvettes contained everything but the substrate or the coenzyme. The temperature of the cuvette compartment was 26° ± 1°C. The application of the method will be discussed in detail under the appropriate sections.

4. The assay of enolase was carried out spectrophotometrically by measuring double bond formation in the reaction yielding phosphopyruvic from phosphoglyceric acid. This results in an increase in absorption measurable at 240 millimicrons (in Colowick and Kaplan, 1955).

Co-factors used were obtained from various commercial sources. Crystalline aldolase and glyceraldehyde phosphate dehydrogenase and the glucostat reagent were purchased from Worthington Biochemical Corporation.

Glucose was estimated by the Nelson-Somogyi (Somogyi, 1952) colorimetric procedure. Lactic acid was determined by the procedure of Barker and Summerson (1941), pyruvic acid

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by the method of Rindi and Ferrari, (1956), and total nitrogen by the method of Johnson (in Umbreit et al, 1951).

RESULTS

The ability of homogenates of *T. spiralis* larvae to degrade glucose is shown in Table I. More than 50% of the glucose utilized may be accounted for as lactic acid. There was no CO_2 nor pyruvate formed in detectable amounts except in experiment No. 6 in which a small amount of pyruvate (0.101 micromole) was produced. Upon withdrawal of HDP, glucose-consumption and lactate-production were decreased. This may be interpreted to mean that the glucose is phosphorylated with the HDP serving as a phosphate reservoir to maintain the level of high-energy phosphate in this system.

These experiments provide only presumptive evidence for operation of a phosphorylative glycolytic pathway comparable to that outlined in other organisms. Therefore, it was necessary to demonstrate a number of the enzymes known to be active in the degradation of glucose to lactic acid by the classical Embden-Meyerhof scheme.

The initial step in carbohydrate-utilization by vertebrate tissues and yeast is the phosphorylation of glucose by a transfer of phosphate from ATP to yield glucose-6-phosphate and ADP (Colowick and Kalckar, 1943). This reaction is catalyzed by hexokinase. That this enzyme is present in the larvae is seen from Table II. In preliminary experiments it was determined that in the absence of fluoride there was no glucose phosphorylated, probably because of pyrophosphatase activity in the homogenate (LePage, 1948).

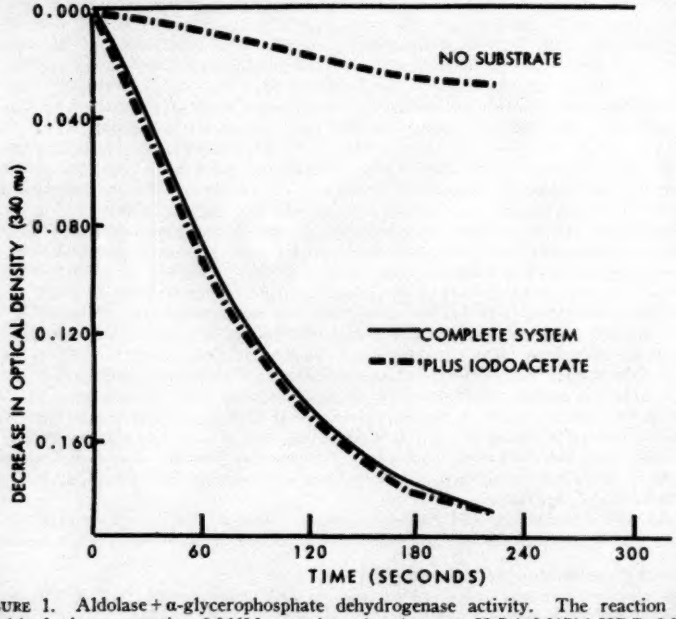

FIGURE 1. Aldolase + α-glycerophosphate dehydrogenase activity. The reaction mixture contained in final concentration, 0.066M potassium phosphate at pH 7.4, 0.017M HDP, 0.000067M DPNH, 0.009M Glycine, Homogenate, and water to 3.0 ml. Optical density readings at 30-second intervals.

Phosphorylation of fructose-6-phosphate to form HDP, splitting of this compound into 2 triose phosphate fragments and the oxidation of one and the reduction of the other is involved in the next series of reactions studied. The enzymes involved include phosphofructokinase, aldolase, glyceraldehyde-3-phosphate dehydrogenase and α-glycerophosphate dehydrogenase. The series of reactions involved may be represented as follows:

1) HDP → dihydroxyacetone phosphate + glyceraldehyde-3-phosphate
(aldolase)
2a) Dihydroxyacetone phosphate + DPNH → α-glycerophosphate + DPN
(α-gly-P dehydrogenase)
Overall reaction: HDP + DPNH → α-gly-P + DPN
2b) Glyceraldehyde-3-phosphate + DPN → Phosphoglyceric acid + $DPNH_2$
Overall reaction: HDP + DPN → PGA + DPNH
(glyceraldehyde-3-phosphate dehydrogenase)

Therefore, by using HDP as substrate and DPN or DPNH as coenzyme, it is possible to obtain a measure of the coupled reaction involving aldolase and α-gly-P dehydrogenase or glyceraldehyde-3-phosphate dehydrogenase.

Reaction 2a, which is shown graphically in Figure 1, has an equilibrium constant of 1.4×10^4 at 22° C and pH 7, and proceeds virtually to completion (In Colowick and Kaplan, 1955). The disappearance of DPNH determined by the decrease in optical density at 340 millimicrons thus becomes a measure of the combined activities of aldolase, α-glycerophosphate dehydrogenase, and phosphotriose isomerase. The data of a typical experiment are plotted in Figure 1. In the absence of

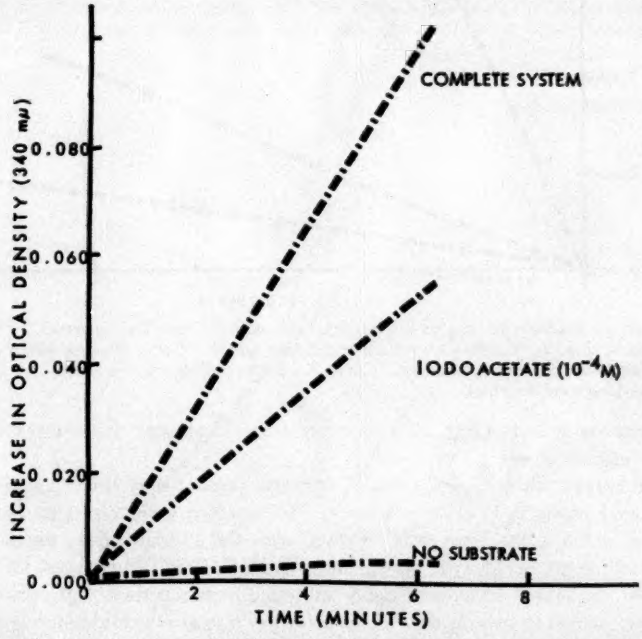

FIGURE 2. Aldolase + glyceraldehyde-3-phosphate dehydrogenase activity. The reaction mixture contained in final concentration 0.02M Tris (hydroxymethyl) aminomethane buffer at pH 7.6, 0.02M Cysteine, 0.009M Glycine, 0.017M HDP, 0.00017M DPN, 0.00017M sodium arsenate, Homogenate, and water to 3.0 ml. Optical density readings at 1-minute intervals.

substrate, the homogenates are still capable of oxidizing DPNH but at a reduced rate. This is probably due to the activity of a DPNH oxidase which has been demonstrated in the tissues of this parasite (Goldberg, 1957a).

The combined activities of aldolase, phosphotriose isomerase, and glyceraldehyde-3-phosphate dehydrogenase, reaction 2b, are shown in Figure 2. Arsenate is added to inhibit the back reaction. There is slight endogenous activity in the absence of HDP. Iodoacetic acid is known to inhibit the activity of the dehydrogenase in this reaction sequence, in most tissues studied. Pre-incubation of the homogenate for 15 minutes with iodoacetate resulted in a 43.6% inhibition of activity (Fig. 2). The lack of effect of 10^{-4} M iodoacetate on α-glycerophosphate

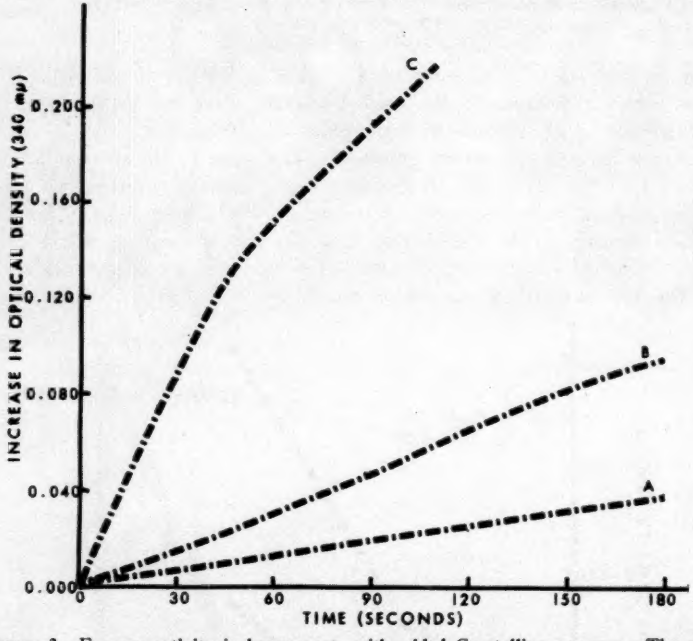

FIGURE 3. Enzyme activity in homogenate with added Crystalline enzymes. The reaction mixture as in Fig. 2. Curve C—crystalline aldolase added. Curve B—crystalline glyceraldehyde-3-phosphate dehydrogenase added. Curve A—no crystalline enzyme added. Optical density readings at 15-second intervals.

dehydrogenase activity (Fig. 1) is as expected and indicates that the reactions proceed as outlined above.

In an extract the complex array of enzymes present may involve substrate, coenzyme, and products in other reactions. To obtain a more direct measure of the activity of aldolase the assay was repeated with the addition of an excess of purified crystalline glyceraldehyde phosphate dehydrogenase. The rate of DPN reduction, then, becomes dependent upon substrate concentration (glyceraldehyde-3-phosphate) which in turn, is dependent upon the rate at which aldolase splits HDP. If instead, excess crystalline aldolase is added, there results an excess of substrate, and DPN reduction becomes a direct measure of glyceraldehyde dehydrogenase

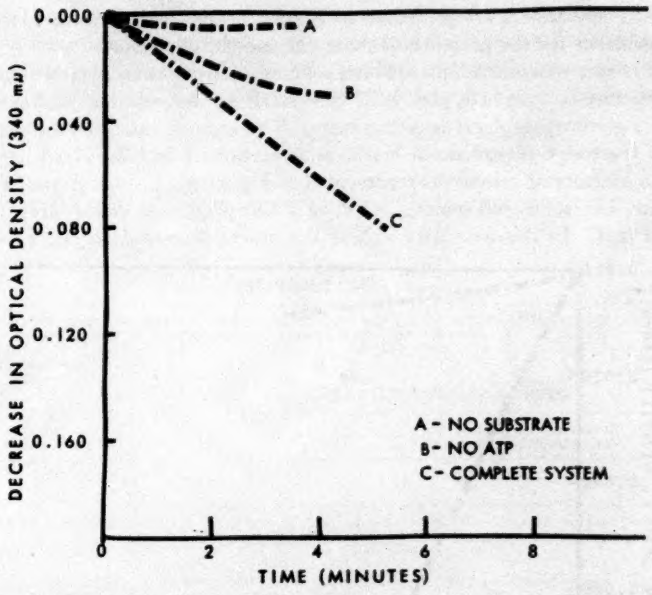

FIGURE 4. Phosphofructokinase activity. The reaction mixture as in Fig. 1 except HDP replaced by 0.017M fructose-6-phosphate and 0.002M ATP. Optical density readings at 1-minute intervals.

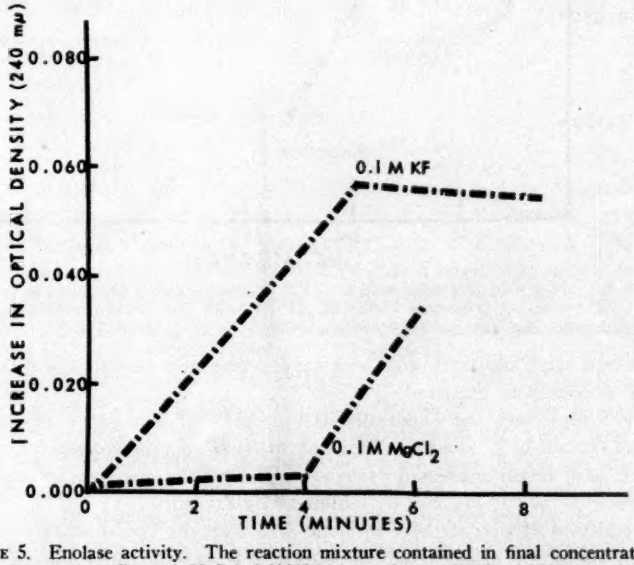

FIGURE 5. Enolase activity. The reaction mixture contained in final concentration, 0.05M potassium phosphate buffer at pH 7.4, 0.0033M magnesium chloride, 0.009M glycine, 0.0067M phosphoglyceric acid, Homogenate and water to 3.0 ml. Optical density readings at 1-minute intervals. $MgCl_2$ or KF added at 4 minutes.

activity. These results are presented in Figure 3, and provide further, more conclusive evidence for the presence of these enzymes in this tissue.

An indirect measure of phosphofructokinase activity was obtained by substituting fructose-6-phosphate plus ATP for HDP in the reaction mixture for the assay of α-glycerophosphate dehydrogenase. The enzyme catalyzes the phosphorylation of fructose-6-phosphate to HDP, and reactions 1 and 2a, above, proceed to provide a measure of activity as represented in Figure 4. Curves A and B indicate that there was some endogenous ATP or other phosphate donor present in the tissue extract. In this assay, as well as the others discussed so far, a 10-minute

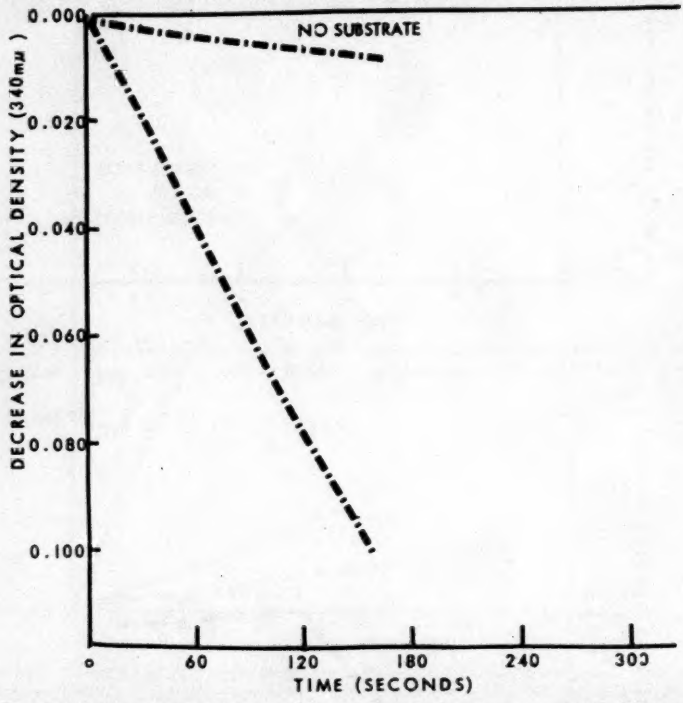

FIGURE. 6. Lactic dehydrogenase activity. The reaction mixture contained in final concentration, 0.033M potassium phosphate buffer at pH 7.4, 0.00033M sodium pyruvate, 0.000067M DPNH, Homogenate and water to 3.0 ml. Optical density readings at 30-second intervals.

pre-incubation with substrate was necessary before any measurable oxidation or reduction of coenzyme occurred.

Figure 5 illustrates the experimental results which provide evidence for the presence of enolase in *T. spiralis*. This enzyme catalyzes the conversion of 2-phosphoglyceric acid to phospho-enol-pyruvate. The results demonstrate the requirement of the enzyme for Mg and its inhibition by fluoride.

The reduction of pyruvic acid to lactic acid with the concomitant oxidation of DPNH, by the action of lactic dehydrogenase is represented in Figure 6. Endogenous activity in this assay is undoubtedly due to DPNH oxidase.

For all of the spectrophotometric assays of enzyme activity the supernatant of

centrifuged homogenates was used. In all cases 100% of the activity of the uncentrifuged homogenate was present in the supernatant. This indicates that, intracellularly, these enzymes are soluble or are localized in particles not sedimented under these conditions. In Table III the activity of the enzymes is summarized.

TABLE I. *Anaerobic glycolysis in* T. spiralis *homogenates.*

Exp. #	Δ Glucose μm	Δ Lactic acid μm	Lactic acid Glucose
1	− 1.73	+ 2.28	1.31
2	− 1.47	+ 1.88	1.28
3	− 1.30	+ 1.72	1.29
4	− 2.25	+ 2.91	1.29
5a	− 0.42	+ 0.58	1.38
6b	− 2.22	+ 2.20	0.99

The complete reaction mixture contained 0.5 μM $MgCl_2$, 1.0 μM K-ATP, 0.5 μM DPN, 5.0 μM K-HDP, 90 μM K-PO_4 pH 7.4, 10 μM glucose. Gas phase N_2, time 60 min.
a. No HDP.
b. Gas phase air.

TABLE II. *Hexokinase activity of* T. spiralis *homogenates.*

	Glucose phosphorylated μM/mg N/30 minutes
Exp. 1	0.562
Exp. 2	0.499

The complete reaction mixture contained in final concentration, 0.02M potassium phosphate buffer at pH 7.4, 0.002M magnesium chloride, 0.001M potassium fluoride, 6 mg glucose, and homogenate. At zero time, 2.4 μM K-ATP added.

TABLE III. *Summary of enzyme activities in* T. spiralis *homogenates.*

Enzyme	Activity*	
	Exp. 1	Exp. 2
Aldolase + α-glycerophosphate dehydrogenase	0.206	0.187
Aldolase + glyceraldehyde phosphate dehydrogenase	0.075	0.067
Aldolase	0.149	....
Glyceraldehyde phosphate dehydrogenase	0.632	....
Phosphofructokinase	0.098	0.072
Enolase	0.621	0.669
Lactic dehydrogenase	0.649	0.689

* μM substrate transformed/minute/mg nitrogen

DISCUSSION

On the basis of the data presented in this report it may be concluded that a mechanism for phosphorylative glycolysis similar to the Emben-Meyerhof scheme of vertebrate tissue is present in the tissues of the larvae of *T. spiralis*. The enzymes involved in this pathway of carbohydrate-degradation have been shown to be similar, at least qualitatively, to those present in vertebrate tissues and yeast (Baldwin, 1952). The significance of such a reaction sequence in an organism is obvious.

Evidence for the Embden-Meyerhoff scheme of phosphorylative glycolysis has been obtained for every parasitic helminth in which this problem has been studied (Bueding and Most, 1953). Therefore, it is not surprising that it occurs in *T. spiralis*. Nevertheless, it has been amply demonstrated that generalizations concerning helminth metabolism can not be made on the basis of results obtained with 1 member of a group (Beuding and Most, 1953; Read, 1956; Read, Rothman and Phifer, 1957) and that each member much be investigated separately.

The significance of the present results in terms of the overall metabolic economy of *T. spiralis* cannot be discussed here profitably. In the homogenate system studied, glucose-degradation to lactic acid is the major pathway of carbohydrate

breakdown. That it is the only path is not to be inferred from these data and in fact, is quite unlikely. It has been reported (von Brand et al, 1952) that fatty acids are produced by intact *T. spiralis*, in vitro, as a result of glycogen degradation. Obviously, in a homogenate system, metabolic defects of one sort or another are certain to be present. Any extrapolation of these results to conditions as they exist in the intact organism or especially to the organism in situ would be extremely tenuous at this time without further experimentation.

SUMMARY

The reaction glucose → lactate has been shown to proceed in suitably fortified homogenates of *Trichinella spiralis* larvae. Identification of a number of the enzymes associated with this process indicates that it follows the Embden-Meyerhof pathway of phosphorylative glycosis. The enzymes which have been demonstrated include hexokinase, phosphofructokinase, aldolase, α-glycerophosphate dehydrogenase, glyceraldehyde-3-phosphate dehydrogenase, enolase and lactic dehydrogenase.

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THE SUSCEPTIBILITY OF LIBERIANS TO THE MADAGASCAR STRAIN OF *PLASMODIUM VIVAX*

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The relative insusceptibility of Negroes to infection with *Plasmodium vivax* has been reported by many observers (Mayne, 1932; Boyd and Stratman-Thomas, 1933; Young, Ellis and Stubbs, 1946; Becker, Kaplan, Read and Boyd, 1946; Young, Eyles, Burgess and Jeffery, 1955). The only exception to these reports of comparative insusceptibility has been that of Butler and Sapero (1947) who found no difference in susceptibility between Negroes and white persons.

As *P. vivax* is rare or absent from large areas of Liberia (Bray, 1957) the susceptibility of Liberians to infection with *P. vivax* was investigated to see if insusceptibility is the dominant factor in the rarity of *P. vivax*. The Liberians infected were living under natural conditions which include hyperendemic malaria due largely to *Laverania falciparum* but also to *P. malariae* and *P. ovale*.

MATERIALS AND METHODS

Thirty Liberian Nationals of all ages, either working at the Liberian Institute or living close by, were exposed to the bites of *Anopheles gambiae* infected with the sporozoites of *P. vivax*.

The Madagascar strain of *P. vivax*[1] employed was maintained in splenectomized chimpanzees and the *A. gambiae* employed were drawn from a laboratory colony.

The method of infection was "normal" in the sense that only 1 to 4 infective *A. gambiae* were allowed to bite a subject. On the other hand the infection in *A. gambiae* was generally heavy. All *A. gambiae* allowed to bite were dissected subsequently to ascertain if the salivary glands contained sporozoites. Three separate batches of subjects were exposed to the bites of infective *A. gambiae* at 3 different times and on 2 of these occasions non-immune whites[2] were similarly exposed as controls.

Following infection thick blood films were stained with Giemsa and examined from all infected subjects from 9 days to 2 months after infection and again when possible 8 to 11 months after infection. Examination was daily in the first period and twice weekly in the second. Where *P. ovale* appeared the presence of this parasite was confirmed in thin quick-dried smears after the method of Bray (1957).

RESULTS

The results are set out in Table I. They show a visible blood infection due to *P. vivax* in only 1 Liberian subject among 30 exposed to infection. The subject U. D. showed *P. vivax* in the blood 14 days after exposure and symptoms of malaria occurred 15 days after exposure. A typical paroxysm occurred 17 days after exposure. The infection was terminated by 1.5 grams of chloroquine base over 24 hours on the 17th day after exposure. No relapse occurred in the 11 months following exposure.

On the 17th day after exposure, gametocytes of *P. vivax* appeared in the blood of U. D. and *A. gambiae* fed at this time became infected. When sporozoites were present these mosquitoes were allowed to bite subject F. O. who failed to show *P. vivax* in the blood.

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[1] The strain of *P. vivax* was received in *Anopheles atroparvus* kindly sent from London by Mr. P. G. Shute.

[2] My gratitude is extended to Dr. Max J. Miller and my wife; the non-immunes.

The subject R. D., who is the 1-year-old son of U. D., failed to show parasites at any time after exposure to infection. The appearance of the parasites in U. D. is worthy of note. Many thin films containing the generation of parasites seen on the 14th and 15th days after sporozoite infection, were studied and the parasite was at first glance thought to be *P. ovale*. Later it was shown definitely not to be by the absence of oval host erythrocytes in quick-dried thin films. The rings were small, compact and the vacuole was well defined, blank and white. The rings resembled piroplasms.

The trophozoites were compact, sometimes 2-nucleated and the vacuole was large and pronounced. The outline was usually round or oval with very few

TABLE I. *The results of infecting Liberian subjects with the sporozoites of the Madagascar strain of* P. vivax.

Subject	Age* in years	Number of infective bites	Parasites† in the blood by months after infection					
			1st month	2nd month	8th month	9th month	10th month	11th month
M.J.M. (control)	A	2	V					
A	A	1	F	F, O	F	F	F	F
S.L.	A	1	–	F	O	F	F	–
H	A	1	F, M	F				
K	A	1	F, M	F	–	F	–	–
C	A	1	F, M	F, M	F	F	F	F
T	A	2	F	F				
B.P.B. (control)	A	1	V					
U.D.	A	3	V	–	–	–	–	F
N	A	2	–	–	–	–	F	F
B	A	4	–	–	–	F	F, O	F
G	A	2	–	F	–	F	F	–
F.O.	A	4	F	F, M				
W	1—10/12	1	–	O	–			
J	2— 4/12	2	F	–	–	–	–	–
O	7	2	–	F	F	F	F	F
S	3	2	F, M	F, M, O	F	F	F	F
M	8	2	F, M	F, M	F	F	F	F
H	5	4	F	–	F	F	–	–
L	3	1	–	F	–	O	–	–
R.D.	1	4	–	–	–	–	–	–
J	A	1	–	–				
V	A	1	–	–				
R	A	1	F	–				
G	A	1	–	–				
S	A	1	–	F				
T	A	1	F	F				
A	A	1	–	F				
P	A	1	–	–				
W	A	1	–	–				
M	A	2	F	F				
G	A	1	–	–				

* A—Adult.
† V—*P. vivax*, F—*L. falciparum*, M—*P. malariae*, O—*P. ovale*.

amoeboid forms. The cytoplasm was collected into a very dense thin line around the periphery of the vacuole. The nucleus of many of the old trophozoites appeared unable to divide, resulting in a number of parasites which filled four-fifths of the cell and displayed a pale cytoplasm with a diffuse pink nucleus which possessed long strands running through it. Although these parasites looked like poorly stained microgametocytes, it is believed that they were degenerating trophozoites.

The schizonts had a pale cytoplasm and large oval nuclei. The number of merozoites was 8–12. The pigment was yellow-brown and scattered in the schizonts.

The host erythrocyte of the trophozoite was enlarged and pale. Heavy staining showed very pale fine Schüffner's dots to be present but these were invisible when stained normally. The host erythrocyte of the schizonts was greatly enlarged and fine pale Schüffner's dots could be seen when normally stained.

The second generation of parasites studied on the 16th and 17th days after infection was completely typical of *P. vivax*. The parasites stained normally and produced 18–28 merozoites.

DISCUSSION

It is obvious that Liberians of all ages are highly resistant to infection with the Madagascar strain of *P. vivax*, as was to be expected from the results of previous work. This factor is obviously the main cause of the absence or rarity of *P. vivax* in Liberia.

The result presented here of a 3% susceptibility is less than the 7.4% susceptibility reported by Young *et al* (1955). Under the conditions of malarial hyperendemicity however, it might be expected that susceptibility would be lower in Liberia than was found in the U.S.A.

The inability to infect F. O. with the line of *P. vivax* taken from U. D. bears out the statement of Young *et al* (1955) that the infection in 1 Negro is not infective to Negro recipients. Similarly inability to infect R. D., the son of the susceptible U. D., would indicate that susceptibility is not a family characteristic though more evidence is needed to establish the point.

The morphology of the first generation of *P. vivax* studied in U. D. is that of *P. wilsoni* Roberts, 1940. It is possible that *P. wilsoni* is merely *P. vivax* slightly modified by sojourn in a Negro host.

SUMMARY

Of 30 Liberian Nationals aged 1–35 years exposed to infection by sporozoites of the Madagascar strain of *P. vivax* only 1 showed a patent infection due to *P. vivax* in the blood. The 1-year-old son of this subject exposed at the same time failed to show a patent infection. The line of *P. vivax* isolated from the susceptible Liberian failed to produce an infection in another Liberian.

The first generation of *P. vivax* studied in the blood of the susceptible Liberian showed the characteristics of *P. wilsoni*. The succeeding generation was typical of *P. vivax*.

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THE EXPERIMENTAL HISTOPATHOLOGY OF THE "TUMOR" ON THE BRAIN OF THE STICKLEBACK CAUSED BY *DIPLOSTOMUM BAERI EUCALIAE* HOFFMAN AND HUNDLEY, 1957 (TREMATODA: STRIGEOIDEA)

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The structure of the "tumor" on the brain of the stickleback fish, *Eucalia inconstans* (Kirtland) caused by *Diplostomum baeri eucaliae*, has been briefly reported (Hoffman and Hoyme, 1957; Hoffman and Hundley, 1957) and consists of an outgrowth of the columnar epithelium of the choroid plexus which surrounds the metacercariae at the postero-lateral aspect of the optic lobes. The present report deals with the experimental development of the "tumor."

No record of a comparable structure in fish could be found and the reader is referred to our earlier paper (Hoffman and Hundley, 1957) for further introduction, and materials and methods.

CONTROL

In the uninfected fish the choroid plexus is a relatively small sack-like structure lined by columnar epithelium, located at the postero-lateral aspect of the optic lobes (Figs. 1, 2). Its anterior border appears to originate from, or at, the posterior side of the nucleated layer of the optic lobe. Its posterior border appears to originate from, or at, the valvula cerebelli; the meninges are very thin. Its function is the secretion of cerebrospinal fluid.

LOCATION OF THE METACERCARIAE

Twenty-eight sticklebacks, experimentally infected for various lengths of time were fixed, serial sectioned, and studied. The following data, converted to percentages, represent a summary of spot checking, i.e. *ca* every fifth section was studied. The worms in all tisues were counted, but for brevity the negative counts are omitted.

1. *15 to 35 minutes*—Subcutaneous tissue 52; gills 22; muscle 13; blood vessels 6; skin 5; viscera 1; oral epithelium 0.5; brain ventricles 0.25. Of 10 worms counted in one fish 9 were in the head region.
2. *1 hour and 40 minutes to 3 hours and 20 minutes*—Subcutaneous tissue 45; muscle 25; skin 13; blood vessels 10; gills 2; fins 2; viscera 2; optic lobes 0.6; large cranial nerves 0.3.
3. *5 to 28 hours*—Choroid plexus 72.2; submeningeal 6.7; eye (sclera and cornea) 6; medulla 5.1; optic nerve 2.3; connective tissue 2.3; just anterior to the optic lobes 2; tissue of optic lobes 2; ventricles of optic lobes 0.7; just posterior to the brain 0.7.
4. *3 days*—Choroid plexus and ventricles 50; tissue of optic lobes 26; submeningeal 10; cerebellum 10; cranial nerves 3; medulla 1.
5. *6 to 8 days*—Choroid plexus and ventricles 47; optic lobes 24; submeningeal 15; cranial nerves 5; medulla 4; cerebellum 2; central aqueduct of brain 2; vitreous chamber of eye 1.
6. *10 days*—Choroid plexus and ventricles 70; optic lobes 24; submeningeal 3; central aqueduct of brain 2; olfactory lobes 0.4.

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7. *22 to 35 days*—Choroid plexus "sack" and ventricles 92; olfactory lobes 5; optic lobes 1; central aqueduct of brain 0.5; vitreous chamber of eye 0.4; cerebellum 0.2; pons 0.2.

8. *50 to 60 days*—Choroid plexus "sack" and ventricles 96; vitreous chamber of eye 2; olfactory lobes 0.6; cerebellum 0.6.

These data indicate that the cercariae penetrate the skin, perhaps in greater numbers in the head region and particularly the gills, migrate through subcutaneous tissue and muscle, and some, at least, enter blood vessels to make their way to the brain region. This is in accord with the findings of the migration of *Diplostomum spathaceum* by Ferguson (1943) and *Posthodiplostomum minimum centrarchi* by Hoffman (1958). A smaller number probably enter the brain directly through the soft parts of the cranium, or by route of the cranial nerves. Subsequently the metacercariae are found under the meninges, in the nervous tissue of the brain itself, particularly the optic lobes, and in the ventricles. Eventually many of them migrate to the choroid plexus at the postero-lateral aspect of the optic lobes where a space exists between the brain and the cranium which allows growth of the "tumor."

EXPERIMENTAL HISTOPATHOLOGY

3 days (Figs. 3, 4): Most of the metacercariae which have reached the brain have localized in 2 main areas, the choroid plexus and the ventricles of the optic lobes. A few remain in the tissue of the optic lobes and just beneath its meninges. There is no evidence of hyperplasia or granulomatous reaction, but resorption of tissue damaged by migrating larvae can be seen. In heavy infections there is considerable hemorrhage postero-lateral to the brain, and behind the eyes.

11 days (Figs. 5–7): At this time the metacercariae, with very few exceptions, are within the choroid plexus or the connecting ventricles of the optic lobes. The columnar epithelium of the choroid plexus has grown to accommodate the growing larvae, and in moderate to heavy infections it extends postero-lateral from the optic lobe. There is a depression in the roof of the cranium which allows freedom for growth of this "tumor-like" structure. The epithelium is thickened and hyperplastic in some places (Fig. 7) and the entire structure is filled with cells which appear to be macrophages (Figs. 6, 7); these surround the worms, each of which may move freely within a restricted area. We assume that the larvae eat these cells because cellular elements can be seen in the ceca and there is no other type of cell in this structure.

42 to 50 days (Figs. 8, 9): There is no appreciable change in pathology, but it is interesting to note that heavily infected fish may survive. The "tumor-like" structure at this time shows no change, but in heavily infected fish there are also many larvae in the tissue of the optic lobes. From the examination of many cross sections it appears that these have migrated through the ependyma into the central white matter. Here there is great loss of brain tissue (Fig. 8) but the ependyma becomes hyperplastic and folds of it partly encircle the worms. Macrophages are also present, particularly in the ventricles. Although there is considerable loss of brain tissue, apparently due to resorption, in heavily infected fish, no impairment of reflexes was noted. When death due to parasites did occur it was probably due to extensive damage and subsequent hemorrhage (Hoffman and Hundley, 1957).

SUMMARY

Stickleback fish, *Eucalia inconstans*, were experimentally infected with the cercariae of *Diplostomum baeri eucaliae*, and the localization of the metacercariae and

the histopathology were studied at intervals up to 50 days. A large majority of the worms localize within the choroid plexus and the optic lobes. The choroid plexus becomes hyperplastic and, in heavy infections, becomes a rather large "tumor-like" structure which extends postero-laterally from the optic lobes; it consists of a substantial amount of columnar epithelium with cells which appear to be macrophages surrounding the worms. Many worms also localize in the inner part of the optic lobes where considerable brain tissue is resorbed followed by hyperplasia of the ependyma and the accumulation of cells appearing to be macrophages.

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EXPLANATION OF FIGURES

FIGURE 1. Frontal section of the brain of an uninfected stickleback. ×32.

FIGURE 2. As Figure 1. ×100.

FIGURE 3. Frontal section of the brain of a 3-day post-infection stickleback. ×32.

FIGURE 4. As Figure 3. ×100.

FIGURE 5. Sagittal section of the brain of an 11-day post-infection stickleback. Note that most of the "tumor" is external to the brain. ×32.

FIGURE 6. As Figure 5. ×430.

FIGURE 7. As Figure 5. Note macrophages and hyperplastic epithelium. ×430.

FIGURE 8. Frontal section of the brain of a 42-day post-infection stickleback. Note that metacercariae are present in the internal white matter of the optic lobes as well as in the "tumor." ×32.

FIGURE 9. Frontal section of the brain of a 50-day post-infection stickleback. Note that a part of the "tumor" is external to the brain. ×100.

ABBREVIATIONS

Cerebel.—Cerebellum
Chor. pl.—Choroid plexus
Epith.—Hyperplastic epithelium
Macro.—Macrophage
Med.—Medulla
Meta.—Metacercaria(e)
Olf. lobe—Olfactory lobe
Opt. lobe—Optic lobe
"Tumor"—Tumor-like cyst of hyperplastic epithelium and macrophages containing metacercariae.

PLATE I

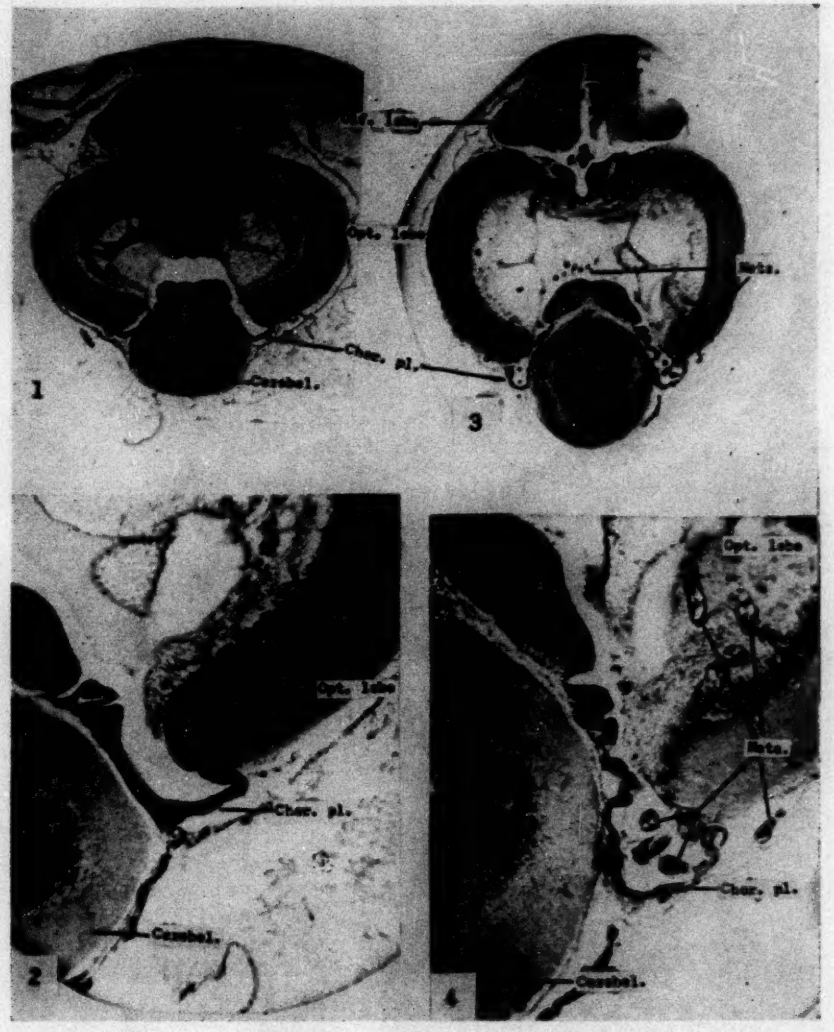
Cyst. Lobe
Cerebel.
Chor. pl.
1
Cyst. Lobe
Meta.
Chor. pl.
3
Cyst. Lobe
Chor. pl.
Cerebel.
2
Cyst. Lobe
Meta.
Chor. pl.
Cerebel.
4

PLATE II

THE LIFE-HISTORY OF *MACRODEROIDES SPINIFERUS* PEARSE, 1924, A TREMATODE OF THE FLORIDA SPOTTED GAR, *LEPISOSTEUS PLATYRHINCUS**

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In determining the life-history of *Paramacroderoides echinus* Venard, 1941, many smaller trematodes were often encountered in the intestine of the gar, *Lepisosteus platyrhincus* De Kay, serving as the final host (Leigh and Holliman, 1956). Frequently small oval cysts were encountered along with those of *P. echinus* in the naturally infected small fishes serving as intermediate hosts of the latter species. Observations in the laboratory indicate that the life-history of the smaller trematode, *Macroderoides spiniferus* Pearse, 1924, parallels that of *P. echinus* in almost every respect. *M. spiniferus* was used by Pearse as the type species for the genus *Macroderoides* and McMullen (1937) used the genus *Macroderoides* as the type genus in establishing the family Macroderoididae. Previously, the only life-history to be reported for a member of this genus is that of *M. typicus* (Winfield, 1929) by McMullen (1935).

MATERIALS AND METHODS

Helisoma duryi, a common pulmonate gastropod of South Florida, was reared in the laboratory and experimentally injected to provide the cercariae used to infect *Gambusia affinis holbrooki* Girard, which were taken from an artificial lake free of gar and were therefore free of the infection. Small gar, 12 to 14 inches in length, were kept unfed in the laboratory for several weeks before being infected and were subsequently fed on small fish taken from the area known to be free of gar.

Measurements and drawings of adults and encysted metacercariae were made on mounted specimens fixed in hot alcohol-formalin-acetic acid mixture and stained in Mayer's carmalum or Semichon's carmine. Cysts were measured alive in saline without flattening. Cercariae were measured after fixation by adding boiling hot 10% formalin to an equal quantity of water and cercariae. It was found that so much shrinkage occurred in dehydrated mounted cercariae that measurements taken were of little value. Measurements taken on the formalin-fixed material were close to those made on living cercariae. Details of the anatomy of the cercaria and sporocyst were worked out primarily on living material aided by the use of neutral red and Nile-blue sulphate. All measurements are given in millimeters.

OBSERVATIONS

Larval Stages

Sporocyst

The eggs of *M. spiniferus* are embryonated and infective at the time of deposition as indicated by their ability to infect the snail host. Since embryonated eggs were never seen to hatch spontaneously, all laboratory infections were established through the ingestion of eggs by laboratory-reared *Helisoma duryi*. Cercariae begin to emerge from the snail host within 30 days at summer temperatures.

Daughter sporocysts, unlike those of *Paramacroderoides echinus* which are situated adjacent to the esophagus and anterior to the liver, are always posterior to the stomach and may eventually replace part of the liver tissue. Failure to migrate results in masses of hundreds of daughter sporocysts marking the original location

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of the mother sporocyst which produced them. The sporocyst mass is a deep orange color due to heavy pigmentation of the paletot layer; the color often shows through the host shell making it possible to determine infection by visual inspection. Attempts to separate attached daughter sporocysts from the parent mass result in the dislodging of the pigmented paletot layer revealing the daughter sporocysts as an almost colorless structure whose body wall contains only a thin scattering of the minute orange pigment granules so conspicuous in the paletot layer.

Pigment granules in the paletot layer are irregular in shape and vary from minute size to 5 microns in diameter. They may be scattered but often appear in compact clusters, as much as 20 microns in diameter, almost filling the cells in which they are located. Since the pigment accumulates progressively with age, it is probably a waste product of the parasite.

As late as 11 months after infection daughter sporocysts still vary greatly in size and number of contained cercariae. The larger sporocysts reach a length of 0.726, measure 0.132 in width and contain as many as 10 mature and nearly mature cercariae as well as many germ balls and immature cercariae in various stages of development. Knob-like thickenings often occur at the ends of the sporocyst.

Cercaria (Figs. 1, 2)

The cercaria of *M. spiniferus* belongs to the Cercariae Armatae group of xiphidiocercariae, possessing a stylet in the oral sucker but no fin-fold on the tail. The entire body cuticle is equipped with minute spines which are most conspicuous in the area adjacent to the oral sucker and become less pronounced toward the posterior end. The tail cuticle is lacking in spines but is thrown into finely sculptured transverse lines when contracted. Body measurements of 30 specimens fixed and measured in alcohol-formalin-acetic acid solution are: length 0.248–0.359 (ave. 0.341); width posterior to acetabulum 0.083–0.110 (ave. 0.090). The mouth is subterminal, located in an ovoid sucker 0.042–0.048 (ave. 0.045) in length by 0.032–0.045 (ave. 0.040) in width equipped with a stylet 0.020 in length (Fig. 3). A well-developed pre-pharynx precedes a muscular pharynx. The slender esophagus bifurcates into 2 rudimentary ceca anterior to the acetabulum. The ventral sucker measures 0.038–0.045 (ave. 0.042) in diameter. This species is unusual in that the penetration glands are unequal in number on the two sides of the body, 4 occurring on one side and 5 on the other. A single small anterior pair resists staining with Nile-blue sulphate and stains more slowly with neutral red than the other glands. In addition to the anterior pair, 3 glands occur on one side and 4 on the other, but the larger number may occur on either right or left side. In 35 cercariae checked for this characteristic, 19 had the larger number on the left side and 16 had the additional gland on the right. The last 2 pairs of glands stain more intensely than the anterior glands with Nile-blue sulphate and neutral red. Ducts proceed anteriorly in a single group on each side around the oral sucker and empty near the distal part of the stylet.

The excretory bladder is a slender I-shaped tube extending from an area just posterior to the acetabulum to caudal pockets at the base of the tail. The bladder wall is covered with a thick layer of cells with coarse granular cytoplasm. Anteriorly, the bladder has 2 small lateral dilatations at the junctions with the primary tubules and a posterior enlargement which discharges through the caudal pockets into a deep ventral groove extending to the posterior end. The primary tubules

pass laterally from the bladder, make several turns or loops and divide into ascending and descending secondary tubules. Active flame cells were rarely seen in the cercaria; sometimes they could be seen at the end of their capillaries in an inactive condition. The density of the parenchyma and penetration glands also hindered the determination of the pattern of flame cells and tubules. However, the clearing of the cytoplasm and increased activity of the flame cells during the first few hours after penetration and encystment made it possible to determine the probable flame cell pattern as $2 \times (3+3+3) + (3+3+3) = 36$ (Fig. 1).

Scattered throughout the body are a number of pale grey oil-like droplets up to 7–8 microns in diameter. Neutral red reveals the presence of numerous, well-distributed gland cells of probable cystogenous function. The tail measures 0.110–0.207 (ave. 0.153) in length and is inserted on the ventral side adjacent to caudal pockets which are lined with cuticula; the posterolateral portions of these pockets are equipped with minute spines.

Metacercaria (Figs. 4–6)

Within 4 to 5 hours after penetration, thin transparent cysts have been formed in muscle tissue. The stylet has broken off from its rounded base and floats freely within the cyst. The excretory bladder has already swollen to fill most of the area posterior to the ventral sucker. The thick layer of cells which covered the tubular bladder of the cercaria has spread thinly over its surface and a clear fluid fills the bladder. In some of the younger forms, muscle fibers around the bladder are still active, so that the bladder alternately expands and contracts, and small refractile granules, less than 1 micron in diameter, are attached to the inner wall of the bladder. Hussey (1941) in studying the development of the bladder of the xiphidiocercaria of *Crepidostomum cornutum* stated that granules and particles of refractive material were contained within the layer of large cells making up the wall of the bladder. Most of the oil-like droplets in the parenchyma still remain and the outlines of penetration glands are still apparent.

After 20 hours of encystment, the most noticeable changes involve the excretory bladder, intestinal crura and the appearance of the parenchyma. The fine granules on the wall of the bladder serve as nuclei for the formation of concretions 2 to 3 microns in diameter most of which are now free in the lumen of the bladder. While individually they are refractile, irregular in shape and pale grey in color, collectively they block light transmission so that the entire bladder appears dark in transmitted light. These concretions are undoubtedly waste products whose sudden appearance is associated with the intense activity of this period.

There is a drastic change in the condition of the parenchyma at 20 hours. In the cercaria the parenchyma was dense and opaque. Now, in contrast, it is composed of large semi-transparent fluid-filled vesicles 35–40 microns in diameter. It is this transparency that makes it possible to work out details of the excretory system that were obscured in the cercarial stage. Visible also are the previously invisible intestinal crura which apparently in the cercaria consist of uncanalized cords. At this stage, neutral red stain reveals that canalization of the crura is complete or nearly complete in most instances. Only a few of the cercarial oil-like droplets remain and the adult pattern of spination is very apparent. Fifteen cysts 20 hours after penetration measured 0.138–0.262 (ave. 0.220) in length by 0.110–0.138 (ave. 0.132) in width.

At the end of the fifth day the cysts have increased in size and a second granular

wall of host origin has been added to that produced by the metacercaria. This wall is fragile and easily separable from the inner cyst. Cysts (15 measured) are now 0.198–0.277 (ave. 0.251) in length by 0.145–0.171 (ave. 0.153) in width. Parenchyma of the metacercaria is still highly vacuolated and transparent. Body spination is pronounced to the level of branching of the intestinal crura and reduced progressively from that point to the posterior end where the spines are small and scattered. There is no change in the appearance of the excretory bladder.

Feeding experiments indicate that metacercarial cysts are infective after 14 days of development in the fish intermediate host at summer temperatures. At that time they have achieved near maximum growth. Cysts 53 days old average 0.256 in length by 0.164 in width; these measurements are not significantly larger than those of the 5-day old cysts. Between 5 and 14 days the principal organs of the reproductive system become sufficiently well developed to achieve rapid maturity after introduction into the final host. The swollen condition of the excretory bladder persists during the entire metacercarial phase. The mature cyst is characterized by 2 cyst walls, an inner thin but tough hyaline wall of parasite origin and an outer fragile connective tissue layer of host origin.

Adult

Comparisons of Florida specimens with Pearse's (1924) description of *M. spiniferus* and Simer's (1929) re-description of the type specimen leaves no doubt concerning the identity of the Florida material which supported Simer's observations with reference to the shape and unequal size of the testes, the lobed condition of the ovary, the size of the pharynx and the position of the ventral sucker.

At 10 days of age, laboratory-reared adults measured 0.83 to 1.30 in length. Specimens 21 days old varied from 1.20 to 1.66 in length. The largest specimen collected from naturally infected gar was 2.37 in length. Average measurements for the 3 largest specimens collected from naturally infected gar were: length 2.24; width 0.38; diameter of oral sucker 0.089; diameter of ventral sucker 0.132; length and width of anterior testis 0.247 by 0.198; length and width of posterior testis 0.293 by 0.210; length and width of ovary 0.153 by 0.130. Living eggs of adult trematodes 10 days old and older were constant in size, measuring 0.048 by 0.019 with an occasional egg departing slightly from these measurements. Pearse (1924) reported the eggs from his specimens to be 0.040 in length. Eggs are fully embryonated at the time of deposition and have clearly visible opercular caps. Eggs from 12-day old adults have been used to infect laboratory-reared snails.

M. spiniferus has been reported previously by Pearse (1924) from the short-nose gar, *Lepisosteus platostomus*, the long-nose gar, *L. osseus* by Simer (1929) and the Florida spotted-gar, *L. platyrhincus* by Bangham (1941). Other hosts reported to harbor this trematode are the yellow bullhead, *Ameiurus natalis* by Pearse (1924) and the brown bullhead, *A. nebulosus* by Pearse (1924) and Bangham (1941). The related *Macroderoides typicus* from *Amia calva* was never found in gar, nor has *M. spiniferus* been found in *A. calva*.

It is of interest to note that *M. spiniferus* resembles *Paramacroderoides echinus* in life-history and time of maturity more than it does its congeneric relative *M. typicus*. While *M. spiniferus* and *P. echinus* use small fishes as intermediate hosts and become egg-producing adults in 10–12 days, *M. typicus* uses tadpoles as intermediate hosts, develops slowly and becomes sexually mature sometime between 49

and 202 days. The life-history of the third member of the genus *Macroderoides*, *M. flavus* Van Cleave 1932, from the pickerel, *Exox niger*, has not been ascertained.

Forty-three of 92 *L. platyrhincus* examined in 1955–57 from waters adjacent to the Tamiami Trail, 20 to 70 miles west of Miami, Florida, were infected with *M. spiniferus*. Infections varied from 1 to 40 trematodes. Of 8 gar, kept in the laboratory unfed for several weeks prior to infection, all but 1 showed infections ranging from 3 to 80 worms which because of size and uniformity of specimens from gar killed at intervals of 4 to 22 days could safely be assumed to have been laboratory induced. General observations indicate that there is a correlation between insufficient or sporadic feeding and rapid loss of many or all parasites and that starvation for several days results in the loss of all parasites. However, this observation has not been confirmed by careful study.

Biology of the Parasite

M. spiniferus cercariae emerge from the snail host after dark, most of them before 9:00 P.M., but show no discernible locomotor response to variations in light intensity. They are weak swimmers and make only slow progress through the water, mostly in the vertical direction. Brief periods of swimming alternate with longer periods of rest during which the cercariae sink slowly to the bottom of the container where they remain for a short time in the floating position. Crawling movements on the substrate do not occur. In the resting position the body is sharply bent into a U-shape with the tail held anteriorly between the 2 halves of the body (Fig. 2). This position is similar to that assumed by *Paramacroderoides echinus* except that the posterior portion of the body is oval rather than expanded into the flattened projections characteristic of *P. echinus*.

When confined snails are heavily infected, most cercariae are trapped in an area of mucus adjacent to the snail host, in which they remain suspended indefinitely with little activity. This mucus is of cercarial origin and consists of strands formed apparently from mucoid discharges of the caudal pockets (Krudenier, 1953). The isolated cercariae are more active, apparently stimulated by sinking to regain the floating position. However, these also possess the mucus strands and may be drawn through the water by a needle to which the trailing invisible strands of mucus adhere. Krudenier (1953) has suggested that the entanglements of cercariae in their accumulated mucus would aid in securing broader contacts with their intermediate hosts or with a substrate in flowing water. When *P. echinus* and *M. spiniferus* cercariae contact fish, the majority of metacercariae become located in the musculature at the bases of caudal and pectoral fins, indicating the effectiveness of the mucus in aiding lodgement of cercariae in the active fins. Penetration, however, can and does occur anywhere on the body surface.

McMullen (1935) reported that the cercariae of *Macroderoides typicus* became entangled in or penetrated mucus secreted by the snail host. It is likely that this mucus also was of cercarial origin. *M. spiniferus* cercariae emerging from lightly infected snails do not produce enough mucus to become massed adjacent to the snail host and are scattered throughout the container. Cercariae exhibit no directional response to the presence of fish or tadpole intermediate hosts until contact occurs. Penetration starts almost immediately with little preliminary wandering from the point of contact and is completed within 5 to 10 minutes. Attachment for the first few minutes is by the ventral sucker allowing the anterior end to engage

in shoveling motions until the anterior end is admitted. Final phases of penetration are characterized by release of the ventral sucker and alternate contractions and extensions of the body until penetration is complete. The tail, which had been directed ventrally and anteriorly during penetration is dropped at the opening of the burrow. Internal migration occurs only when the initial attachment occurs on a fin. There, penetration of the integument is always followed by migration toward the base of the fin where encystment occurs in muscle tissue. Attachment to eyes is followed by attempted penetration of the cornea. Partial penetration occurs, but cercariae have never been seen to penetrate completely into the eye.

The cercariae of *M. spiniferus* will accept a wide variety of intermediate hosts. While *Gambusia affinis holbrooki* Girard is the principal host because it is most numerous, cercariae penetrate and encyst normally in other fish, *Mollienesia latipinna* Le Seur, *Heterandria formosa* Agassiz and *Fundulus* sp. They also encyst normally in the tadpoles of *Rana pipiens sphenocephala* Cope and *Rana grylio* Stejneger.

DISCUSSION

The most unique characteristic of *C. M. spiniferus* is the lack of 1 member of the second and more medial pair of what was primitively 5 pairs of penetration glands. This condition has already been noted by Leigh and Holliman (1956) in *C. Paramacroderoides echinus* and also occurs in the cercaria of a new and undescribed species of the same genus from Florida gar. No record of this asymmetry has been found for other xiphidiocercariae. The only life-history of a member of the genus *Macroderoides* reported previously is that of *M. typicus* (Winfield, 1929) by McMullen (1935). The cercariae of *M. typicus* and *M. spiniferus* are similar in many respects, but 5 complete pairs of penetration glands were seen in *C. M. typicus*, and the possession of a fin-fold on the tail places that cercaria in the group Cercariae Ornatae. It is surprising that 2 species of the same genus should have cercariae assigned to different groups. Luhe's (1909) separation of the Cercariae Ornatae from the Cercariae Armatae on the basis of presence or absence of a fin-fold on the tail has been followed by some workers, but the grouping does not appear to be of any significance phylogenetically. This is further indicated by the fact that *Paramacroderoides echinus* and the undescribed species of the same genus from Florida gar have cercariae which are similar in most respects but differ with reference to the possession of a fin-fold on the tail. Cable (1956) has also pointed out the artificial nature of the widely used system of cercarial classification.

The conspicuous refractile droplets in the parenchyma have been reported for other xiphidiocercariae. McMullen (1935) reported them in *C. Macroderoides typicus* and referred to them as concretions. Byrd and Reiber, 1940, reported and figured 3 species of xiphidiocercariae with what appear to be the same structures although they refer to them as "cystogenous glands that appear as clear droplets of oil." Miller (1936) states that *C. pachycystata* had "small oil-like droplets bluish in color" distributed throughout the body. Leigh and Holliman (1956) reported numerous orange-yellow droplets in *C. Paramacroderoides echinus*. These structures are better characterized as droplets rather than concretions, since they exhibit the flexibility of an oil droplet under pressure. The droplets in *M. spiniferus* and *P. echinus* are still abundant after encystment is complete; therefore, they are not likely to play any direct role in cyst-formation. The clearing of the parenchyma during

the first few hours after encystment is less related to the disappearance of the droplets than to a massive vacuolization of the previously dense, opaque parenchyma.

The appearance of minute granules in the excretory bladder during the first 6 hours of metacercarial existence and their rapid growth into conspicuous concretions is one of the most striking aspects of metacercarial transformation. These were noted by McMullen (1935) and Leigh and Holliman (1956) for the metacercariae of *M. typicus* and *P. echinus*, respectively. They occur in a wide variety of trematode metacercariae. The concretions in mature metacercariae vary in size and while most tend towards a spherical shape, many are irregular. They are semi-solid in contrast to the fluidity that marks the oil-like droplets in the parenchyma of the cercaria. Minute granules serve as nuclei around which 1 to several concentric layers of material are added. A union of small concretions early in the formation process may result in the construction of irregular bodies of multiple origin (Fig. 7).

SUMMARY

Helisoma duryi, a common pulmonate snail of South Florida is frequently infected with a xiphidiocercaria which penetrates and encysts in the muscle tissue of several species of small fishes, the most abundant being *Gambusia affinis holbrooki*. Tadpoles may also serve as intermediate hosts. Metacercariae in fish become infective in 14 days and after being eaten by the Florida spotted gar, *Lepistosteus platyrhincus*, become sexually mature in the small intestine in 10–12 days. The adults, identified as *Macroderoides spiniferus* Pearse, 1924, were found in 47% of the gar examined.

Eggs of this trematode are embryonated and infective when deposited but hatch only after ingestion by the snail host. Daughter sporocysts are located adjacent to the intestine and produce cercariae approximately 30 days after infection of the snail host.

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PLATE I

EXPLANATION OF PLATE

(All figures except 2 and 7 drawn with the aid of a camera lucida)

FIGURE 1. Cercaria of *Macroderoides spiniferus*, ventral view.
FIGURE 2. Sketch of cercaria showing resting position.
FIGURE 3. Stylet of cercaria.
FIGURE 4. Metacercaria, 20 days old, from living specimen.
FIGURE 5. Metacercaria, 20 days old, fixed and stained specimen, ventral view.
FIGURE 6. Metacercaria, encysted.
FIGURE 7. Sketches showing variation in size and shape of bladder concretions in metacercaria.
FIGURE 8. Adult, ventral view.
FIGURE 9. Embryonated egg of adult.

RESEARCH NOTE

HOST-SPECIFICITY OF *TRYPANOSOMA BARBARI*

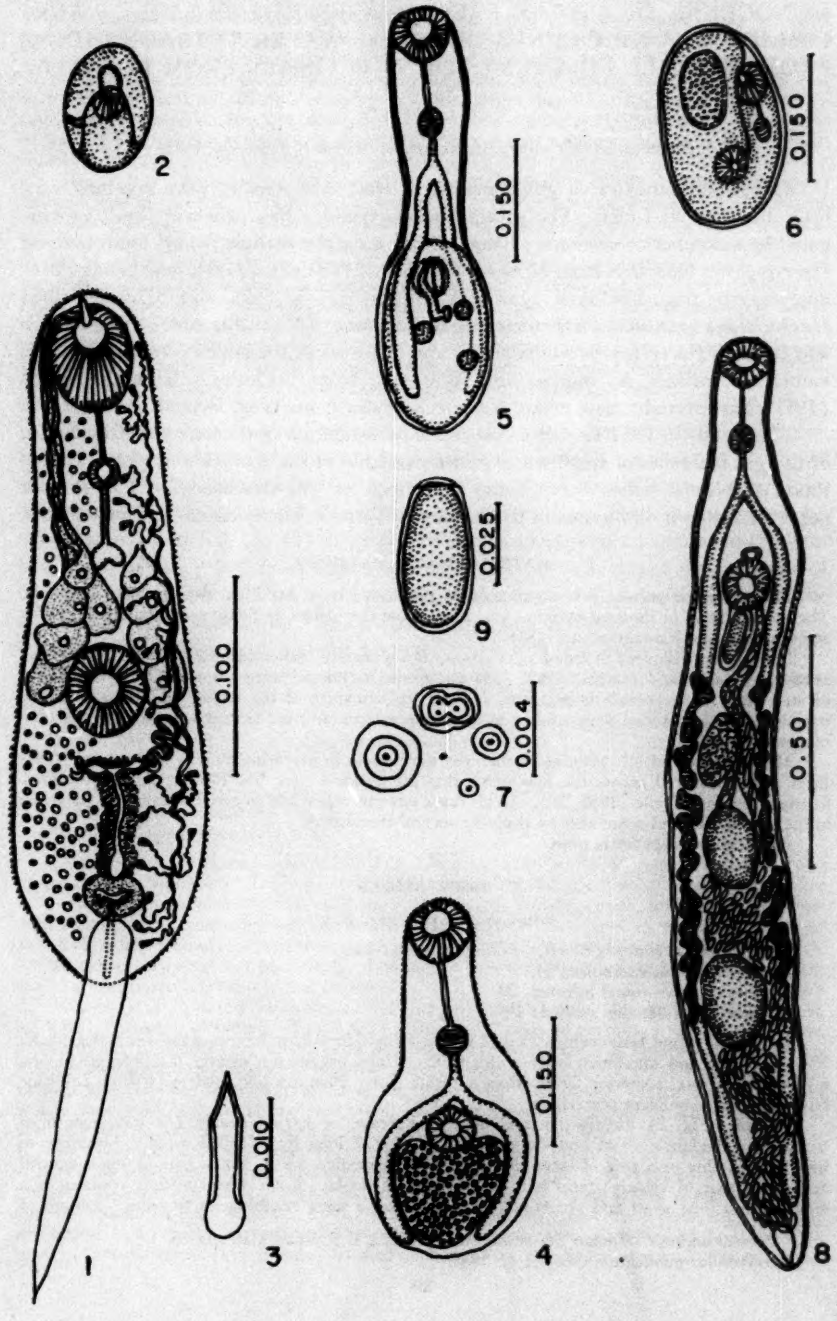
The host specificity of urodelean trypanosomes has been investigated by Lehmann (1952, J. Parasitol. **38**: 550–553) who determined that injections of blood stream forms of *Trypanosoma barbari* could produce an infection only in the natural host, *Taricha t. torosa;* other species including *Taricha granulos twittyi* and *Diemyctylus viridescens* were refractory to infection. Barrow et al (1952), J. Tenn. Acad. Sci. **28**: 190) using blood stream and invertebrate forms of *T. diemyctyli,* found that only the natural host (*D. viridescens*) could be infected, while other aquatic Urodela and Salientia were resistant. Finally, Lehmann (1957, J. Parasitol. **43**: 75) infected *Taricha g. granulosa* with cultural but not blood stream forms of *T. ambystomae.*

Taricha t. torosa infected with *Trypanosoma barbari* were obtained from Roland's Pond, Marin Co., Calif.; in addition 9 *Rana aurora draytoni* and 6 *Hyla regilla* were taken from the same source. Five larval *Dicamptodon ensatus* were collected from the stream leading into Roland's Pond and 7 larvae of the same species were captured in Dutch Bill Creek, Sonoma Co. Twelve *Taricha granulosa twittyi* and 7 *T. rivularis* were found in both Marin and Sonoma Counties while 3 *T. sierrae* were obtained from Amador Co.

The experimental species were divided into approximately 3 equal groups and three methods were used in attempting to infect them with *Trypanosoma barbari:* 1) Animals were placed in wire cages and immersed in Roland's Pond; the animals were checked daily for leeches. In order to ascertain infections, the ectoparasites were removed, dissected, and Giemsa-stained smears prepared of the cecal contents. 2) Leeches were removed from infected *Taricha torosa* and allowed to feed upon experimental animals for a period of 24 hours; following this period, smears were prepared from the cecal contents and examined for hemoflagellates. 3) Leeches removed from infected animals were macerated in 0.7% saline and 1.0 ml of the mixture injected subdermally into the gular region of experimental animals. Prior to injection, the mixture was examined microscopically for the presence of flagellates. Before being subjected to experiments, the vertebrates were examined for hemoflagellates by means of a Giemsa-stained thin blood smear. Following infection experiments, the animals were examined daily over a period of 30 days, by both saline wet mounts and Giemsa-stained thin smears. On the 30th day, each animal was destroyed and Giemsa-stained smears made of heart blood, liver, spleen and lung. NNN cultures were prepared from all species of Taricha and 2 of the *D. ensatus.*

Regardless of the method of injection, natural or artificial, an infection was not found in any of the experimental animals. Giemsa-stained smears of peripheral blood were consistently negative as were smears of the viscera and the NNN cultures. As a result of the evidence obtained in these and previously reported experiments, it appears that *Trypanosoma barbari* (both blood stream and vector forms) is restricted to a single species of host, *Taricha t. torosa;* it is speculated that this restriction is physiological rather than ecological.—DONALD L. LEHMANN, *Department of Zoology, College of the Pacific, Stockton, Calif.*

PLATE I



MONOGENEA OF INDIAN FRESH-WATER FISHES. VII. *BIFURCOHAPTOR*, A NEW GENUS OF FRESH-WATER TETRAONCHINAE FROM THE GILL FILAMENTS OF TWO FISHES FROM LUCKNOW

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Trematode parasites of gill filaments in fresh-water fishes have received very little attention in India. The group Gyrodactyloidea has, however, been investigated by a number of workers. Price (1938) gave the earliest record by describing *Dactylogyrus moorthyi* from Mysore. Thapar (1948) described a new genus *Paradactylogyrus* from Lucknow. Jain (1952, '52a, '55, '57, '57a, and '57b) described *Haplocleidus gomtius, Thaparocleidus wallagonius, Urocleidus notopterus, Mizelleus indicus, Dactylogyrus multispiralis* and 3 species of the genus *Neodacytlogyrus*, namely *N. calbasi, N. indicus* and *N. cotius*, from Lucknow. Recently Baugh (1957) has erected a new genus *Neogyrodactylus* from West Bengal.

The author in 1952 started a systematic investigation of monogenetic trematodes of the gill filaments of fresh-water fishes available in the Lucknow region. It was found that most fishes carry heavy infections of gill trematodes. The present paper describes a new genus in the subfamily Tetraonchinae, based on 2 new forms.

MATERIALS AND METHODS

Fishes for the present investigation were purchased from the local fish market, Lucknow. The identification of the host material was made from the papers of Shaw and Shabbeare (1937) and Chauhan and Ramakrishna (1953).

For an easy recovery of trematodes from gill filaments of fishes Mizelle's (1936, '38) refrigeration technique and Hargis' (1952, '53) chloretone technique were employed. For the study of structures of taxonomic importance, such as the armature of the haptor and the copulatory complex, the trematodes were studied in glycerine mounts and the sketches made with the help of a camera lucida.

Measurements of all chitinized structures were made in glycerine mounts and curved structures were measured across the line subtending the greatest arcs described by those structures, as suggested by Mizelle (1936, '38). In all cases attempt was made to give the range in variation in the size of different structures by studying several trematodes.

All measurements are in mms.

OBSERVATIONS

Bifurcohaptor indicus n. g., n. sp.

Host: Mystus vittatus (Bloch); Family Notopteridae.
Number of fishes examined: 24.
Number of fishes found infected: 20.
Number of trematodes studied: 10.

Diagnosis: Stout tetraonchids (Fig. 1), measuring 0.55–0.82 in length, with width at cephalic lobes 0.05–0.06 and maximum body width 0.085. Head organs not clearly divisible into lobes. Eye-spots, 2 pairs, posterior larger than anterior pair. Pharynx, oval, 0.034 × 0.035. Intestine bifurcate, crura confluent posteriorly.

Haptor (Fig. 2) forceps-like and deeply bifurcate, occupying nearly 1/3 total length of trematode. Peduncle short and stout. Haptor 0.2–0.27 long by 0.15–0.29 wide. Armature of haptor consisting of a pair of large dorsal anchors, a median dorsal bar, a pair of small ventral anchors, a pair of ventro-lateral bars and 6 pairs of hooks. Each dorsal anchor consists of a stout base, a long shaft and recurved point; each has a wing reaching up to point. Length of

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dorsal anchor 0.16–0.2. Base of each dorsal anchor (Fig. 3) bears 3 chitinized plates, *left, right* and *median* according to position each one occupies. Plates measure in greatest diameter: left 0.023–0.026, median 0.014–0.017 and right 0.024–0.027. Dorsal bar (Fig. 3) stout cubical body wth blunt irregular projections at the corners, measuring 0.041–0.045 × 0.033–0.036 in greatest diameters. Ventral anchors situated at the 2 lateral extremities of the haptor, each with bifurcate base, stout shaft, fine recurved point, and wing reaching to the point. Each ventral anchor 0.016–0.025 long with width of base 0.012. Dorsal anchors 8 to 9 times larger than ventral anchors. Each ventro-lateral bar a straight shaft with a slight depression at one end and 0.035–0.042 long. The 6 pairs of hooks are all similar in shape and size, 0.01–0.012 in length. Each consists of a base, a short shaft, a sickle-shaped portion and an apposable piece.

Testis (Fig. 1) median, spindle-shaped, intercecal, measuring 0.09–0.12 × 0.017–0.021. Vas deferens long but no well defined vesicula seminalis. A pair of prostate glands (Fig. 4) present, their ducts opening at base of cirrus. One of prostate glands (pr. 1) contains a highly granular fluid and other (pr. 2) contains a hyaline fluid. Copulatory complex (Fig. 4) consists of a chitinized tubular cirrus and an accessory piece, free at base but articulating distally. Cirrhal gland globular, joining at point of articulation of cirrus and accessory piece. Cirrus 0.07–0.075 long. Accessory piece 0.021–0.025 long. Ovary ovoid, pretesticular and intercecal in position, measuring 0.074–0.082 × 0.03–0.036. Ova large at the anterior end and smaller at posterior end of ovary. Vagina sinistral, chitinized, opening into globular receptaculum seminis by slender vaginal tube. Single egg (Fig. 5) present in some specimens, triangular, length of 3 sides; 0.035, 0.042 and 0.02. Vitellaria densely spread over body from pharynx to cecal union.

This trematode is assigned to the subfamily Tetraonchinae Monticelli, 1903 due to the presence of 2 pairs of anchors and absence of squamodiscs in haptor. It shows some affinity with the genera *Murraytrema* Price, 1937 and *Thaparocleidus* Jain, 1952 in possessing 3 bars in the haptor, but differs from both in the structural peculiarities of haptor, i.e., (1) large size of dorsal anchors as compared to the ventral anchors, (2) great development of dorsal anchors, (3) great development of dorsal bar to support dorsal anchors, (4) presence of well defined plates on the bases of dorsal anchors, and (5) forcipate shape of the haptor.

It is evident, therefore, that the present form is different from the known genera of the subfamily Tetraonchinae and is assigned to a new genus, *Bifurcohaptor*, indicating the shape of haptor.

Bifurcohaptor giganticus n. sp.

Host: Mystus seenghala (Sykes); Family Notopteridae.
Number of fishes examined: 18.
Number of fishes found infected: 1.
Number of trematodes studied: 8.

Diagnosis: Very large tetraonchids, 3.25–3.76 long, with width at cephalic lobes 0.3 and maximum body width 0.6. Three pairs of head organs (Fig. 6), made up of bunches of smaller tufts, joined by a common duct on each side. Pharyngeal glands present, taking a bright stain with haematoxylin, consisting of minute glandular organs. Two pairs of eye-spots, posterior pair with a larger number of melanistic granules. Pharynx, oval, measuring 0.16 × 0.15. Intestine bifurcate, ceca confluent posteriorly. Outlines of intestine are obliterated in some specimens due to dense vitellaria present in that region.

Haptor (Fig. 7) deeply forcipate, 0.8–1.2 long and 0.6–0.85 wide. Peduncle short and stout, 0.3 wide. Armature of haptor (Figs. 8 & 9) consists of 2 pairs of anchors, a median dorsal bar, a pair of ventro-lateral bars and 6 pairs of hooks. Dorsal anchors of immense length giving forceps-like appearance to haptor. Ventral anchors situated at the 2 ends of haptor and individually articulated to ventro-lateral bar of their own side. Each dorsal anchor consists of stout base, a long shaft and strong recurved point. Each provided with a pair of wings, situated at posterior part of anchor and reaching to the points. Each anchor 0.8–1.125 long and 0.14 wide at base. Width of shaft near base 0.1, in middle 0.11 and near the origin of wings 0.045. Base of dorsal anchor bears 3 chitinized plates (Fig. 9), as in genotype *B. indicus;* plates measure in greatest diameter: left 0.115, median 0.085 and right 0.1. Three sharp spines at points of dorsal anchors, measuring 0.06, 0.035 and 0.015. Dorsal bar (Fig. 8) stout cubical body with irregular projections at 4 corners, measuring 0.14–0.15 × 0.16–0.17 in greatest diameters, articulating with bases of dorsal anchors at its 2 ends. Ventral anchors (Figs. 7 & 8) situated at 2 ends of haptor.

Each consists of bifurcate base, short shaft and sharply recurved point. Each anchor provided with double wings, not reaching to point, and is 0.045–0.051 long, 0.03–0.035 wide at base. Size proportion of dorsal and ventral anchor about 20:1. Each ventro-lateral bar (Fig. 8) with spatula-like distal end, 0.165–0.19 long. Six pairs of marginal hooks, ventral, all similar in shape and size; 0.011–0.013 long. Each hook sickle-shaped, consisting of base, shaft, sickle-shaped portion and an apposable piece.

Testis median, elongated and pear-shaped, intercecal, measuring 0.25–0.31 × 0.15–0.19. Vas deferens long and stout, opening into highly chitinized vesicula seminalis. Cirrus sigma-shaped, highly chitinized, joined to vesicula seminalis proximally by stout duct and distally to accessory piece. A pair of prostrate glands also open at base of cirrus, both containing highly granular fluid. One of prostate glands (pr. 2) has 2 small internal pouches, both filled with granular fluid. Cirrus 0.23–0.26 long, 0.025 wide at base. Cirrhal gland, small elongated organ opening at base of accessory piece. Accessory piece (Fig. 10, a. p. 1, a. p. 2) consists of 2 irregular elongated pieces, articulated together, each measuring 0.11–0.121 and 0.09–0.12 in length, providing a forceps-like copulatory apparatus.

Ovary median, reniform and pretesticular, measuring 0.2–0.23 × 0.1–0.13. Vagina (Fig. 11) sinistral, tubular and highly chitinized with wide mouth, continued into chitinized oval receptaculum seminis. Mouth of vagina measures 0.035; receptaculum seminis 0.045 × 0.05. Oviduct long and coiled. No egg observed in specimens studied. Vitellaria densely spread from pharynx to confluence of intestinal ceca. Vitelline ducts join in middle of body just anterior to ovary.

This species is assigned to the genus *Bifurcohaptor* n. g. due to the forcipate shape of the haptor, the presence of well defined plates at the bases of dorsal anchors, presence of a median dorsal and a pair of ventro-lateral bars in the haptor, very large size of the dorsal anchors and non-articulation of the cirrus and accessory piece at the base.

It can be clearly distinguished from the genotype, *B. indicus*, in several important characteristics: (1) presence of 3 sharp cuticular spines on the points of each dorsal anchor, (2) ratio in the size of dorsal and ventral anchor is 20: 1, whereas in the genotype it is 8–9: 1, (3) morphology of the copulatory complex, and (4) the average size of the trematode is about 5 times that of the genotype.

The specific name refers to the large size of the trematode. As far as the author could determine, it is the largest fresh-water tetraonchid known.

Genus *Bifurcohaptor* n. g.

Diagnosis.—Tetraonchinae, with several pairs of head organs and 2 pairs of eye-spots. Intestine bifurcate and crura confluent posteriorly. Haptor deeply forcipate in shape. Two pairs of anchors, dorsal and ventral, dorsal anchors several times larger than ventral anchors. Conspicuous plates present on bases of dorsal anchors. Ventral anchors situated at the two extremities of haptor. Bars, 3, a stout median dorsal and a pair of ventro-lateral bars situated at extremities of haptor. Hooks, 12. Gonads in middle to anterior third of body. Copulatory complex consists of cirrus and accessory piece, non-articulate at base. Vagina sinistral. Single egg. Parasites of gill filaments of fresh-water fishes.

Type species: Bifurcohaptor indicus.

Key to the species of *Bifurcohaptor*

Dorsal anchors stout, nearly 20 times as large as ventral anchors, bear 3 sharp spines on the points ..... *B. giganticus*

Dorsal anchors about 8–9 times as large as ventral anchors, devoid of spines on points. ..... *B. indicus*

SUMMARY

A new genus *Bifurcohaptor* is described, based on 2 new species, *B. indicus* and *B. giganticus*, from the gill filaments of some Indian fresh-water fishes. The outstanding characteristics of this tetraonchid are (1) forcipate shape of the haptor, (2) very large size of dorsal anchors as compared to ventral anchors, 8 to 20 times, (3) presence of 3 plates on bases of dorsal anchors, and (4) presence of 3 bars in the haptor.

ACKNOWLEDGMENTS

The present investigation was carried out in the Department of Zoology, University of Lucknow, India, under the guidance of Professor M. B. Lal. I am grateful to him for constant encouragement and helpful criticism throughout the course of the work. I am also grateful to the authorities of Lucknow University for laboratory facilities and kind permission to publish the present work as part of my approved Ph.D. thesis. My acknowledgments to Government of India are due for the award of a research training scholarship to carry out the present work.

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EXPLANATION OF PLATES

Abbreviations: ***a.e.***, anterior eye-spots; ***a.p.***, accessory piece; ***cir.***, cirrus; ***cir.gl.***, cirrhal gland; ***d.a.***, dorsal anchor; ***d.b.***, dorsal bar; ***e.***, egg; ***h.***, hook; ***h.o.***, head organ; ***hp.***, haptor; ***int.***, intestine; ***l.p.***, left plate; ***m.p.***, median plate; ***m.v.***, mouth of vagina; ***ov.***, ovary; ***p.e.***, posterior eye-spots; ***ph.***, pharynx; ***ph.gl.***, pharyngeal glands; ***pr.***, prostate glands; ***r.p.***, right plate; ***r.s.***, receptaculum seminis; ***sp.***, spine; ***t.***, testis; ***v.a.***, ventral anchor; ***vag.***, vagina; ***v.d.***, vas deferens; ***vit.***, vitellaria; ***vit.d.***, vitelline duct; ***v.l.b.***, ventro-lateral bar; ***v.s.***, vesicula seminalis; ***v.t.***, vaginal tube.

FIGURE 1. *Bifurcohaptor indicus*, entire worm, dorsal view.
FIGURE 2. " " , haptor, ventral view.
FIGURE 3. " " , elements of haptoral armature.
FIGURE 4. " " , copulatory complex etc.
FIGURE 5. " " , egg.
FIGURE 6. *Bifurcohaptor giganticus*, entire worm, ventral view.
FIGURE 7. " " , haptor, ventral view.
FIGURE 8. " " , base of ventral anchor.
FIGURE 9. " " , elements of haptoral armature.
FIGURE 10. " " , copulatory complex, prostate glands, vesicula seminalis and part of vas deferens.
FIGURE 11. " " , vagina and receptaculum seminis.

RESEARCH NOTE

HUNTERELLUS THEILERI FIEDLER (ENCYRTIDAE, CHALCIDOIDEA) PARASITIZING AN AFRICAN *HYALOMMA* TICK ON A MIGRANT BIRD IN EGYPT

The writers recently established, from evidence from migrating birds at Cairo, Egypt, that immature stages of a typically African tick, *Hyalomma rufipes*, are frequently carried to Egypt and probably to Europe and Asia on northward migrants (Hoogstraal and Kaiser, 1958, Ann. Ent. Soc. Amer. **51**: 12–16). On 20 March 1957 a specimen (HH4524, host 398) of European wheatear, *Oenanthe o. oenanthe* (Linnaeus) (identified by Dr. Melvin A. Traylor, Chicago Natural History Museum) was trapped at Abu Rawash near Cairo. The same day, a fully engorged nymph of *H. rufipes* dropped from the bird. Approximately 1 month later, on 23 April 1957, 50 females of *Hunterellus theilerae* Fiedler, 1953 (Encyrtidae, Chalcidoidea) (identified by Dr. B. D. Burks of the United States National Museum) emerged from the nymph through a single small, round hole in the posteromedian groove of the dorsal integument. The tick died without molting. The emergence hole was surrounded by a discolored area appearing to be dry, oozed blood and the integument of at least the posterior third of the tick formed an empty shell.

The bird host was en route from somewhere north of the Zambesi River in southern Africa to Europe or Asia. The tick and its hymenopterous parasites might have been carried much farther north if the bird host had not been trapped in Egypt.

H. theilerae was originally described from specimens emerging from nymphs of *Hyalomma truncatum* Koch and *Rhipicephalus oculatus* Neumann in Southwest Africa and South Africa, respectively (Fiedler, O. G. H., 1953, Onderstepoort J. Vet. Res. **26**: 61–63). No other records have been published. The present data are of interest because (1) of the large number of females only that emerged from a single nymph, (2) the addition of *Hyalomma rufipes* to the known hosts, (3) the record of time elapsed between tick host detachment and wasp parasite emergence, and (4) the unique example of what is probably a fairly common distribution factor for this and possibly other parasites of ticks.—MAKRAM N. KAISER AND HARRY HOOGSTRAAL, *Naval Medical Research Unit No. 3, American Embassy, Cairo, Egypt.*

PLATE I

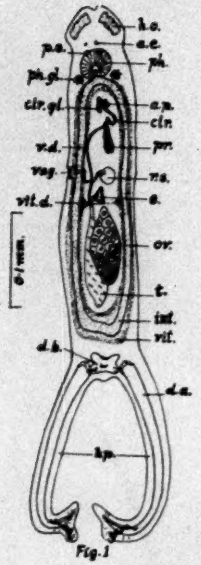


Fig. 1

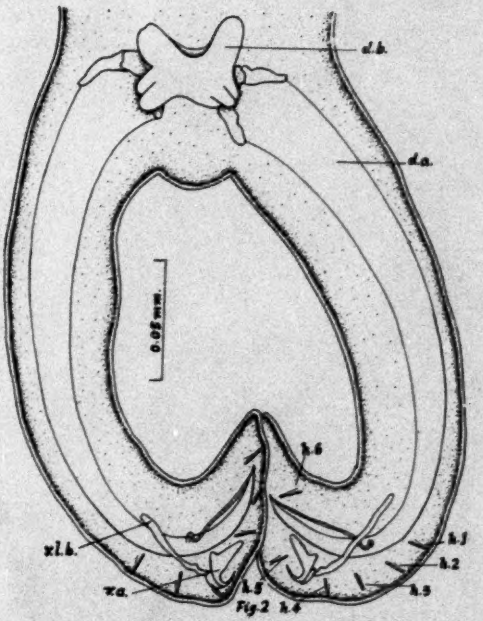


Fig. 2

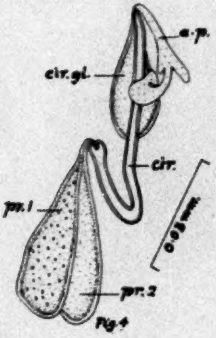


Fig. 4

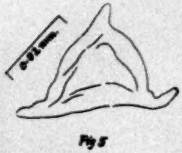
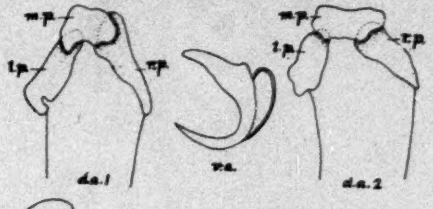


Fig. 5



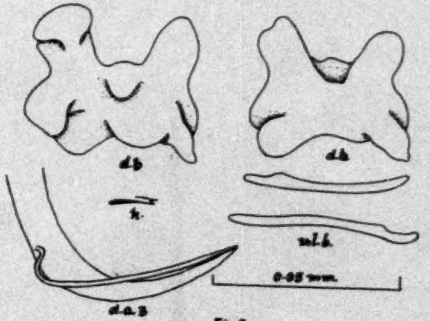
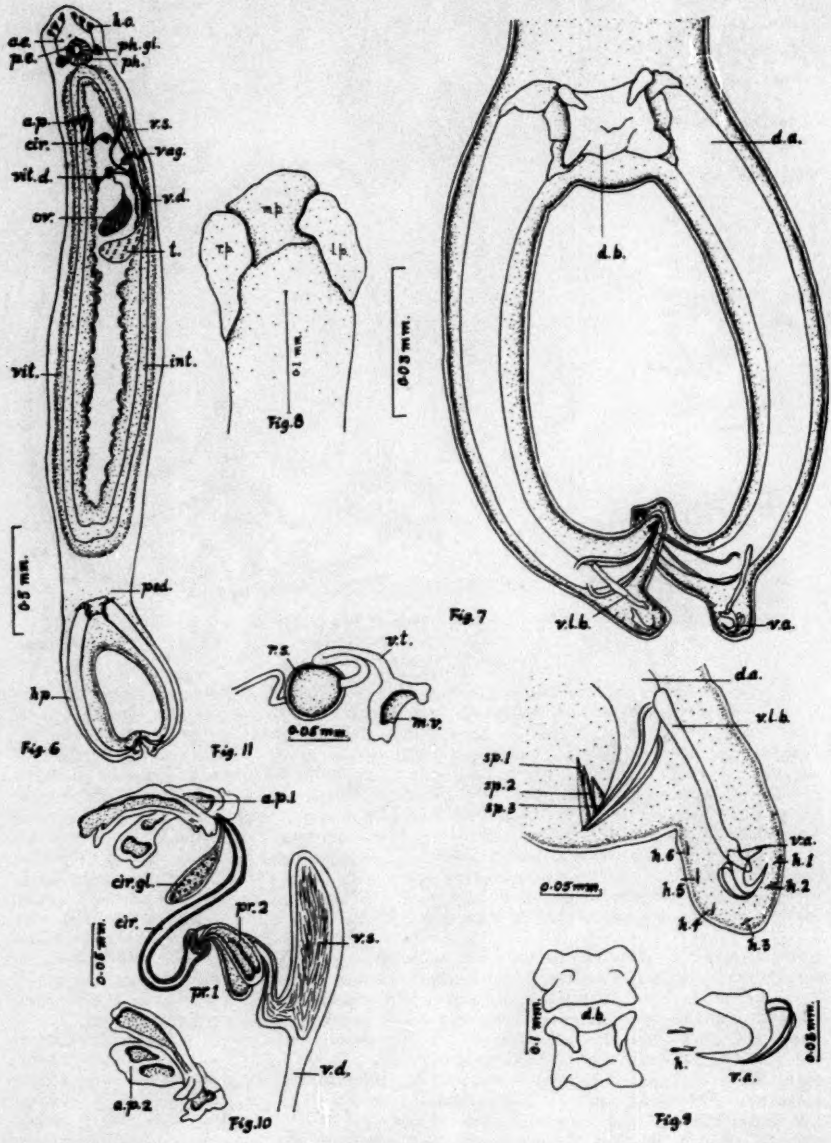


Fig. 3

PLATE II


l.a.
a.s.
p.s.
ph.gl.
ph.
a.p.
v.s.
cir.
vag.
vit.d.
v.d.
ov.
t.
vit.
int.
0.5 mm.
pad.
hp.
Fig. 6
m.p.
l.p.
r.p.
0.1 mm.
0.03 mm.
Fig. 8
d.a.
d.b.
v.l.b.
v.a.
Fig. 7
r.s.
v.t.
m.v.
0.05 mm.
Fig. 11
a.p.1
cir.gl.
cir.
pr.2
pr.1
v.s.
0.05 mm.
a.p.2
v.d.
Fig. 10
d.a.
v.l.b.
sp.1
sp.2
sp.3
v.a.
h.6
h.1
h.5
h.2
h.4
h.3
0.05 mm.
0.1 mm.
d.b.
h.
v.a.
0.05 mm.
Fig. 9

A NEW FAMILY CONTAINING TWO NEW GENERA OF CYCLOPOID COPEPODS PARASITIC ON STARFISHES

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The copepods described below were collected in West Africa and Madagascar by the senior author during 1954–55. They were recovered from the filtered sediment after washing the starfishes in 5% ethyl alcohol in sea water.

The field work was made possible by a fellowship awarded the senior author by the John Simon Guggenheim Memorial Foundation and the subsequent laboratory study was aided by a grant from the National Science Foundation.

STELLICOMITIDAE n. fam.

Body minute, oval, swollen dorsally, the segmentation not evident. Head region comprising nearly half of the body. Abdomen minute. First antenna 3- or 4-jointed. Second antenna 4-jointed without an exopodite. Labrum and labium forming a very short sucking apparatus, and often showing slight sexual dimorphism. Mandible a simple spine-like process with an adjacent seta representing the palp. First maxilla with 2 branches. Second maxilla 2-jointed. Maxilliped 4-jointed.

Swimming legs biramous, with the endopodites usually reduced to a single podomere, and with the exopodites 3-jointed except for the 1-jointed exopodite of leg 1. Leg 5 a single small podomere. Leg 6 present or absent in the male.

Caudal ramus minute, variable in form. Two oval egg sacs, each with a single embryo. Spermatophores oval.

Parasites on the body surface of Asteroidea.

Stellicomes n. gen.

Female: Body minute, oval, swollen dorsally, without external segmentation. Abdomen minute. Rostrum absent. First antenna 4-jointed with chitinized areas along the posterior edges of the podomeres. Second antenna 4-jointed, the last podomere ending in a truncated process and a spine. Mouth area with the labrum and the labium forming a very short sucking apparatus. Mandible a simple spine-like process, with an isolated seta near its base representing the palp. First maxilla with 2 branches, each with 3 setae. Second maxilla 2-jointed, the distal podomere ending in a spinose pad. Maxilliped 4-jointed, elongate, the last podomere ending in a seta and a weak claw bearing a subterminal tooth.

Swimming legs biramous with the rami highly modified, having the spine and setal formula as given in the description below. Leg 1 small, each ramus consisting of a single podomere. Leg 2 elongate, with both rami 3-jointed. Legs 3 and 4 shorter than the second. Leg 3 with a 3-jointed exopodite and a 2-jointed endopodite. Leg 4 with a similar exopodite and a 1-jointed endopodite. Each of the first two exopodite podomeres of legs 3 and 4 with a prominent, well-chitinized, dentate spine. Leg 5 a single podomere with 3 setae. Leg 6 absent.

Caudal ramus minute with 4 slender setae and a long terminal seta.

Two oval egg sacs, each with a single embryo.

Male: Body form and appendages like the female. Slight sexual dimorphism in the mouth area. Spermatophores oval.

Type species: Stellicomes tumidulus n. sp.

(The generic name is derived from *stella,* a star, and *comes,* a companion.)

Measurements given below are in microns unless otherwise stated.

Stellicomes tumidulus n. sp.

Type material: 500 copepods from the body surface of 6 starfish, *Protoreaster lincki* (Blainville), in 0.5 meters at low tide on coral sand, Pointe Lokobe, Nossi-Bé, Madagascar, June 13, 1955. Holotype female, allotype, and 125 paratypes deposited in the collection of the Institut de Recherche Scientifique de Madagascar at Tananarive, and the same number of para-

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types in the Muséum National d'Histoire Naturelle at Paris, in the United States National Museum, and in the authors' collection.

Other specimens: 675 copepods from the surface of 17 *Poraster superbus* (Möbius), in 4 meters on a sandy bottom, off the tip of Pointe Lokobe, Nossi-Bé, June 23, 1955; 525 from 2 *Pentaceraster mammillatus* (Audouin), in shallow water at low tide, Nosy Komba, June 20, 1955; and 825 from 4 *Culcita schmideliana* (Retzius) in 1 meter at low tide among coral, N. Tanykely, near Nossi-Bé, July 19, 1955.

Female: Body (Figs. 1, 2, and 3) minute, compact, and swollen dorsally. Total length 321 (300–348), greatest width 236 (216–264), based on 10 specimens. In dorsal view (Fig. 1) the greatest width at the level of the second maxillae. Head and thorax fused, without external evidence of segmentation. An internal chitinized framework visible through the dorsal cuticula. Genital segment and abdomen without segmentation and short, tapering rather abruptly. In lateral view (Fig. 2) the anterior part of the head not swollen dorsally, but the rest of the head and thorax very tumid. Genital segment and abdomen not swollen.

Egg sacs (Fig. 1) oval, 126×90, each containing a single egg.

Rostrum absent. First antenna (Fig. 4) with 4 podomeres, but the third podomere apparently incompletely subdivided to form 2 parts. First podomere with both edges conspicuously chitinized and with a single anterior seta. Second podomere with a posterior chitinized edge, numerous setae, and a single aesthetask. Third podomere with several short chitinized areas along its posterior edge and with several anterior setae. Fourth podomere with several setae and a subterminal aesthetask.

Second antenna (Fig. 5) with 4 podomeres, the last incompletely subdivided. First podomere unarmed, the second with a single seta, the third with a row of spines along 1 edge, and the fourth bearing 4 small setae and a large subterminal spine, and terminating in a truncated process having a minutely bifurcate tip.

Mouth area (Fig. 6) with the labrum and the labium having a conspicuous internal chitinized framework and only slightly projecting to form a poorly developed sucking apparatus. Mandible (Fig. 7) a simple spine-like process. A single isolated seta in the region of the base of the mandible representing the palp. First maxilla (Fig. 7) minute, consisting of 2 branches, the inner with 3 spine-like setae, the outer with 3 slender setae. Second maxilla (Fig. 8) large, composed of 2 podomeres, the first broad and unarmed, the second slender and elongate, with a spinose pad 14×9 at its tip. Maxilliped (Fig. 9) long and slender, composed of 4 podomeres, the first unarmed and elongate, the second elongate with a single seta, the third only ¼ the length of the second and armed with 3 minute spinules, the fourth elongate and slender with a row of very small spinules along 1 edge and bearing terminally a seta and a weakly chitinized claw with a subterminal tooth.

Swimming legs much modified. Leg 1 (Fig. 10) with both exopodite and endopodite consisting of a single podomere, the exopodite armed with 6 setae, the endopodite with 1. Second leg (Fig. 11) with both rami 3-jointed, the exopodite longer, its second podomere with a single inner seta, the third podomere mucronate and bearing 4 setae; endopodite with a single small seta on the first podomere and a single seta and a group of delicate hairs on the last podomere. Third leg (Fig. 12) with the exopodite 3-jointed and with similarly arranged setae, but with the addition of a strongly chitinized, dentate, recurved distal spine and a row of about 9 smaller spines on the first podomere, and a similar spine and row of 4 spines on the second podomere; third podomere elongate, mucronate, weakly chitinized, and fringed along the inner edge. Endopodite 2-jointed with 2 groups of long fine hairs on the inner edge. Distal podomere with 2 delicate setae. Leg 4 (Fig. 13) similar to leg 3, but with only 2 short setae on the last exopodite podomere, and the last endopodite podomere terminating in a single seta whose articulation is obscure. A prominent outer basipodite seta present on all 4 legs.

Spine and setal formula (though difficult to express for such modified legs) as follows:

	leg 1		leg 2		leg 3		leg 4	
	exp	end	exp	end	exp	end	exp	end
1st podomere	6	1	0:0	0:1	1:0	0:0	1:0	1
2nd podomere			0:1	0:0	1:1	2	1:1	
3rd podomere			4	1	4		2	

Leg 5 (Fig. 14) with a single podomere about 12×10, its articulation obscure, bearing three distal setae, the anteriormost slightly stouter than the other two. Leg 6 absent.

Caudal ramus (Fig. 15) minute, 16×10, tapering distally, having a slender inner distal projection, and bearing four outwardly directed slender setae, and a large terminal seta 84 long, with its base retracted into the ramus.

Color in life translucent, eye red, red globules in the intestinal region, egg sacs opaque in reflected light. The red eye spot of a single embryo showing in each ripe egg sac.

Male: Body form as in the female. Total length 320 (312–324), greatest width 260 (252–264), based on 3 specimens. Head and thoracic appendages like those of the female. Mouth area (Fig. 16) differing in details, the inner chitinized framework more angular. Spermatophores (Fig. 17) 34×24. Caudal ramus as in the female. Color like that of the female.

Stellicomes guineensis n. sp.

Type material: 325 copepods from the body surface of 2 starfish, *Oreaster clavatus* Müller and Troschel, dredged in 4 fathoms about 5 miles west of Cape Sierra Leone, Sierra Leone, November 16, 1954. Holotype female, allotype, and 80 paratypes in the Muséum National d'Histoire Naturelle at Paris, the same number of paratypes in the collection of the Institut Français d'Afrique Noire at Dakar, in the United States National Museum, and in the author's collection.

Female: Body form (Fig. 18) similar to the preceding species. Cuticula soft and often somewhat distorted in preserved specimens. Total length 358 (348–372), greatest width 262 (252–264), based on 6 specimens. An internal chitinized framework showing through the dorsal cuticula. Segmentation of the head and thorax not visible. Genital segment and abdomen indistinctly separated by a fold of cuticula. In side view the thorax swollen dorsally as in *S. tumidulus.* A few long delicate hairs scattered over the dorsal surface of the abdomen and the genital segment.

Egg sacs (Fig. 18) oval, 182×106, each containing a single embryo. Rostrum absent. First antenna (Fig. 19) probably 4-jointed, but the third podomere suggesting a weak subdivision into 2 parts. Armature and chitinization of the posterior edge very similar to that of *S. tumidulus,* but the aesthetask on the second podomere absent. Second antenna (Fig. 20) like the preceding species, but the seta on the second podomere very delicate and the podomere itself shorter.

Mouth area (Fig. 21) with groups of spine-like projections in the sucking cavity formed by the labrum and the labium. Mandible (Fig. 22) slender and more elongate than in *S. tumidulus,* with a similar adjacent seta representing the palp. First maxilla (Fig. 22) similar to *S. tumidulus.* Second maxilla (Fig. 23) and the maxilliped (Fig. 24) like those of the preceding species.

Leg 1 (Fig. 25) similar to *S. tumidulus,* but the setae of the exopodite naked and with 2 unequal, weakly articulated setae on the tip of the endopodite. Leg 2 (Fig. 26) similar also, but the seta on the first endopodite podomere here a broad, strongly chitinized spine. Leg 3 (Fig. 27) similar, but with longer rows of spines on the first 2 exopodite podomeres, an additional row on the last exopodite podomere, and 1 of the 4 setae on this podomere short, slender, and naked. The 2 setae on the last endopodite podomere distinctly plumose. Leg 4 (Fig. 28) like that of *S. tumidulus.* The outer basipodite seta absent on the last 3 legs, and instead at that point a row of minute spines.

Spine and setal formula like that of the preceding species except for the addition of a small seta on the end of the endopodite of leg 1. Leg 5 (Fig. 29) 18×13, somewhat withdrawn into the thorax, its articulation indistinct, with three terminal setae. Leg 6 absent.

Caudal ramus (Fig. 30) similar to that of *S. tumidulus.*

Color in life as in *S. tumidulus.*

Male: Body form as in the female. Total length 372 (360–384), greatest width 304 (288–312), based on 3 specimens. Appendages as in the female. Mouth area (Fig. 31) with the teeth in the sucking cavity formed by the labrum and the labium more prominent than in the female. Internal chitinized framework of the mouth area quadrangular in outline, rather than oval as in the female. Caudal ramus as in the female. Spermatophores like those of *S. tumidulus.* Color as in the female.

Remarks.—*S. guineensis* may be distinguished from *S. tumidulus* by its more elongate egg sacs, by the presence of 2 unequal setae on the tip of the endopodite of leg 1, by the arrangement of spines on the exopodite of leg 3, and by details of the second antenna and the mouth area.

Onychopygus n. gen.

Female: Body minute, oval, greatly swollen dorsally, without external segmentation. Abdomen minute. Rostrum absent. First antenna 3-jointed, with chitinized areas along the posterior margins of the podomeres. Second antenna 4-jointed, terminating in 2 recurved claws. Mouth area with the labrum and the labium forming a very short sucking apparatus. Mandible a single spine-like process with a seta near its base representing the palp. First maxilla with 2 branches, each with 3 setae. Second maxilla 2-jointed, the second podomere forming a recurved claw with an inner subterminal tooth. Maxilliped 4-jointed, the fourth podomere bearing a stout seta and a recurved claw with a single tooth.

Swimming legs biramous, much modified, with the spine and setal formula as given in the description below. Leg 1 small, with 1-jointed rami. Leg 2 with an elongate, 3-jointed exopodite and a very small 1-jointed endopodite. Legs 3 and 4 with 3-jointed exopodites (each of the first 2 podomeres bearing a well-chitinized, recurved, dentate spine) and minute 1-jointed endopodites. Leg 5 a single podomere with 3 setae. Leg 6 absent.

Caudal ramus minute, curving outwardly and ending in a recurved spine. Three setae near the middle of the ramus and a small hair at a notch on the outer edge. Two oval egg sacs, each with a single embryo.

Male: Body form and appendages as in the female. Sexual dimorphism in the mouth area not evident. Leg 6 consisting of three setae. Spermatophores oval.

Type species: Onychopygus impavidus n. sp.

(The generic name is derived from ὄνυξ, a claw, and πυγή, a rump.)

Onychopygus impavidus n. sp.

Type material: 350 copepods from the body surface of 2 starfish, *Acanthaster planci* (Linné), in 0.5 meters at low tide at N. Tanykely, near Nossi-Bé, Madagascar, June 22, 1955. Holotype female, allotype, and 85 paratypes deposited in the collection of the Institut de Recherche Scientifique de Madagascar at Tananarive, and the same number of paratypes in the Muséum National d'Histoire Naturelle at Paris, in the United States National Museum, and in the authors' collection.

Female: Body form (Fig. 32) oval, the abdominal region hidden in dorsal view. In lateral view the dorsal region of the head and thorax greatly swollen (Fig. 33). Total length 436 (408–468), greatest width 253 (240–264), based on 10 specimens. Head, thorax, and abdomen without visible segmentation. Genital segment and abdomen very small.

Egg sacs (Fig. 32) oval, 182×120, each containing a single embryo.

Rostrum absent. First antenna (Fig. 34) with 3 podomeres, but the third podomere indistinctively subdivided into 3 parts. The chitinized areas along the posterior edge and the arrangement of the setae similar to *Stellicomes,* with a single aesthetask on the last podomere.

Second antenna (Fig. 35) with 4 podomeres, the first unarmed, the second and third with rows of spinules as indicated, and the last with 2 terminal claws and 2 subterminal spines.

Mouth area (Fig. 36) with the labrum and labium only slightly projecting to form a weak sucking apparatus. A conspicuous internal chitinized framework as in *Stellicomes.* Mandible (Fig. 36) a slender, attenuated spine. A single isolated seta representing the palp near the base of the mandible. First maxilla (Fig. 37) as in *Stellicomes,* with the setae relatively short. Second maxilla (Fig. 38) with 2 podomeres, the first broad and unarmed, the second forming a slender recurved claw with an inner subterminal tooth. Maxilliped (Fig. 39) composed of 4 podomeres, the first unarmed, the second with an inner spine, the third short with a minute peg-like spine, the fourth bearing an inner distal seta and a recurved claw 17 long with a slender tooth on its concave edge.

Swimming legs much modified. Leg 1 (Fig. 40) with 1-jointed rami, but the exopodite indistinctly divided. Armature as in *S. guineensis,* with 2 unequal setiform processes on the end of the endopodite. Leg 2 (Fig. 41) with a 3-jointed exopodite, the second podomere bearing an inner seta, and the last podomere much attenuated and having 5 setae. Endopodite consisting of a single short podomere bearing terminally 2 setae. Leg 3 (Fig. 42) with the exopodite 3-jointed, the first 2 podomeres armed as in *S. guineensis,* but the last podomere attenuated in a long spine-like process and bearing 3 setae. Endopodite minute, 1-jointed, bearing 2 setae. Both rami with membranous edges. Leg 4 (Fig. 43) with the exopodite similar to the third leg, but the last podomere having a more elongate spine-like process and armed with only 1 seta. Endopodite minute and armed with a single seta. Membranous edges as in leg 3. An outer basipodite seta present on all 4 legs.

Spine and setal formula as follows:

	leg 1		leg 2		leg 3		leg 4	
	exp	end	exp	end	exp	end	exp	end
1st podomere	6	2	0:0	2	1:0	2	1:0	1
2nd podomere			0:1		1:1		1:0	
3rd podomere			5		3		1	

Leg 5 (Fig. 44) minute, the single podomere with an indistinct articulation and armed with 3 setae. Leg 6 absent.

Caudal ramus (Fig. 45) minute, 20 long, curving outwardly and ending in a recurved spine, creating the appearance of a claw (hence the generic name). Three outwardly directed setae in the middle of the ramus and a minute hair located at a subterminal outer notch. Edges of the ramus membranous.

Color in life in transmitted light translucent, eye red, egg sacs opaque.

Male: Body form as in the female. Total length 398 (372–420), greatest width 264 (252–276), based on 10 specimens. Head and thoracic appendages like those of the female. Mouth area not significantly different from that of the female. Leg 6 (Fig. 46) consisting of 3 setae. Spermatophore (Fig. 47) 76×50. Caudal ramus as in the female. Color like that of the female.

Onychopygus may be separated from *Stellicomes* by the recurved claw instead of the spinose pad on the second maxilla, by the 1-jointed rather than 3-jointed endopodite in leg 2, by the short recurved terminal seta in place of the long straight terminal seta on the caudal ramus, and by the presence of a sixth leg in the male of *Onychopygus.*

The new siphonostome family Stellicomitidae is allied to the Cancerillidae Sars 1915 and to the Micropontiidae Gooding 1957, both of which also have prehensile second antennae, in contrast to the non-prehensile type of the remaining siphonostome families. In these 3 families the siphon is absent or reduced to a very short extension of the labrum and the labium.

The families may be distinguished as follows:

	Cancerillidae	Micropontiidae	Stellicomitidae
external segmentation	present	present	absent
first antenna	6–8 podomeres	13 or 14 podemeres	3 or 4 podomeres
leg 4	rudimentary or absent	present, but exopodite only	present
leg 5	1-jointed or absent (?)	2-jointed	1-jointed
sexual dimorphism	differences in body form, first antenna, and form of swimming legs	differences in body form, first antenna	absent except for minute differences in the mouth area

The differences between the Stellicomitidae and the other known siphonostome families are of such a fundamental nature that it seems best to us to establish this new family to receive the 2 new genera from asteroids. With our present knowledge of the Siphonostoma the family constitutes a distinct natural group. Undoubtedly much remains to be learned of the interrelationships and evolution among these copepods.

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Sars, G. O. 1915 An account of the Crustacea of Norway with short descriptions and figures of all the species. Vol. 6, Copepoda Cyclopoida, parts 9 and 10, Ascomyzontidae (concluded), Acontiophoridae, Myzopontiidae, Dyspontiidae, Artotrogidae, Cancerillidae. Pp. 105–140.

Explanation of Plates

All figures were drawn with the aid of a camera lucida. The letters following each figure title refer to the scale at which the figure was drawn.

Plate I

Stellicomes tumidulus n. gen., n. sp., female

Figure 1. Dorsal (A).
Figure 2. Lateral (A).
Figure 3. Ventral (A).
Figure 4. First antenna (B).
Figure 5. Second antenna (C).

Plate II

Stellicomes tumidulus n. gen., n. sp., female

Figure 6. Labrum and labium (C).
Figure 7. Mandible and first maxilla (C).
Figure 8. Second maxilla (C).
Figure 9. Maxilliped (C).
Figure 10. First leg (D).
Figure 11. Second leg (D).

Plate III

Stellicomes tumidulus n. gen., n. sp., female

Figure 12. Third leg (D).
Figure 13. Fourth leg (D).
Figure 14. Fifth leg (C).
Figure 15. Caudal ramus, dorsal (C).

same, male

Figure 16. Labrum and labium (C).
Figure 17. Genital segment with spermatophores and abdomen, dorsal (B).

Plate IV

Stellicomes guineensis n. gen., n. sp., female

Figure 18. Dorsal (A).
Figure 19. First antenna (B).
Figure 20. Second antenna (C).
Figure 21. Labrum and labium (C).
Figure 22. Mandible and first maxilla (C).
Figure 23. Second maxilla (C).

Plate V

Stellicomes guineensis n. gen., n. sp., female

Figure 24. Maxilliped (C).
Figure 25. First leg (D).
Figure 26. Second leg (D).
Figure 27. Third leg (D).
Figure 28. Fourth leg (D).
Figure 29. Fifth leg (C).

Plate VI

Stellicomes guineensis n. gen., n. sp., female

Figure 30. Caudal ramus, ventral (C).

same, male

Figure 31. Labrum and labium (C).

Onychopygus impavidus n. gen., n. sp., female

Figure 32. Dorsal (A).
Figure 33. Lateral (A).
Figure 34. First antenna (C).
Figure 35. Second antenna (C).

Plate VII

Onychopygus impavidus n. gen., n. sp., female

Figure 36. Labrum and labium (C).
Figure 37. Mandible and first maxilla (C).
Figure 38. Second maxilla (C).
Figure 39. Maxilliped (C).
Figure 40. First leg (D).

Plate VIII

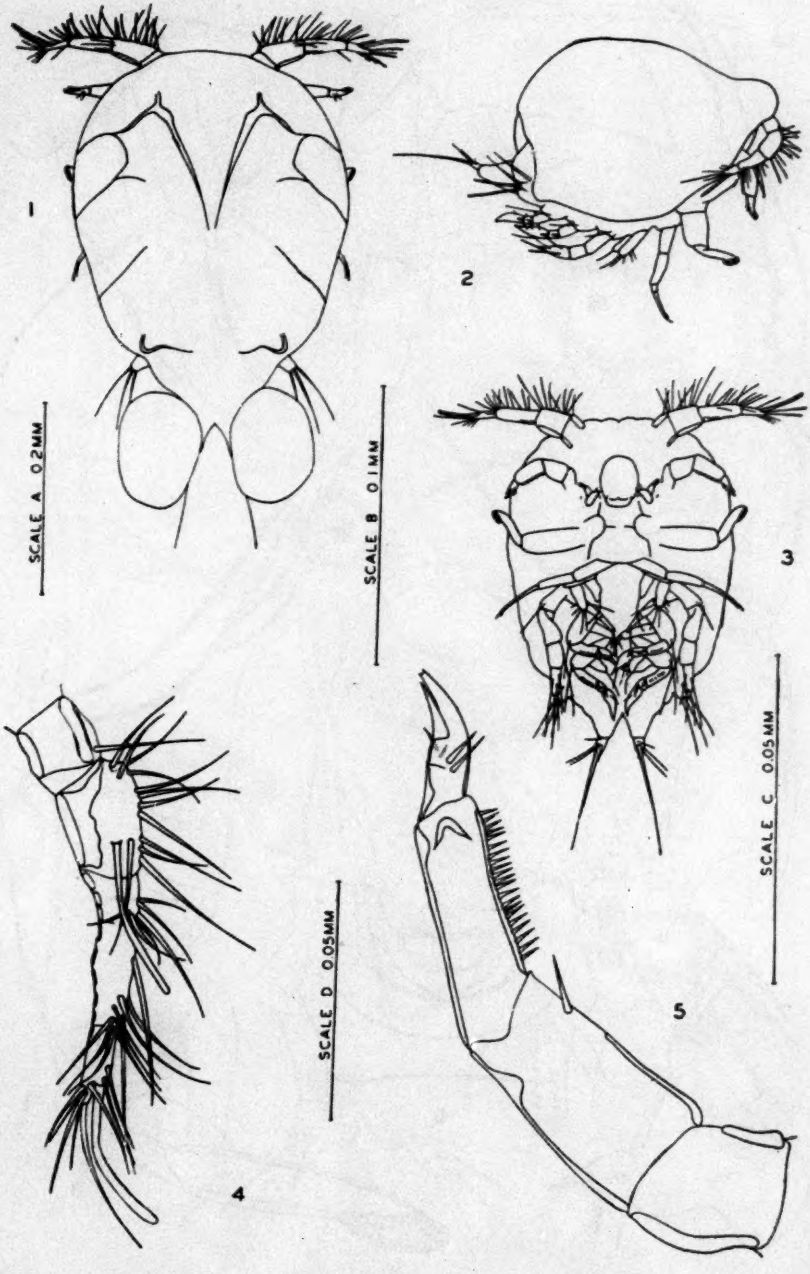
Onychopygus impavidus n. gen., n. sp., female

Figure 41. Second leg (D).
Figure 42. Third leg (D).
Figure 43. Fourth leg (D).
Figure 44. Fifth leg (C).
Figure 45. Caudal ramus, dorsal (C).

same, male

Figure 46. Fifth and sixth legs (C).
Figure 47. Spermatophore (B).

PLATE I



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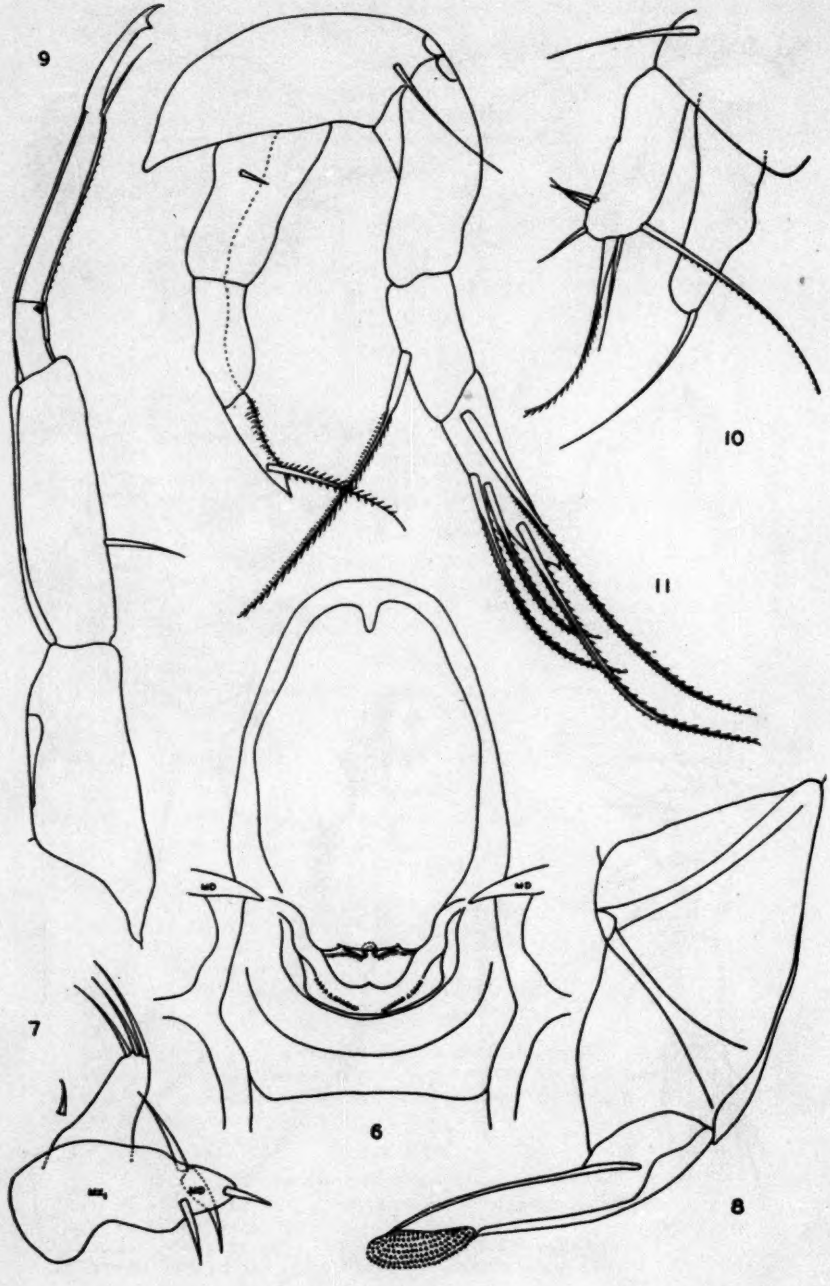

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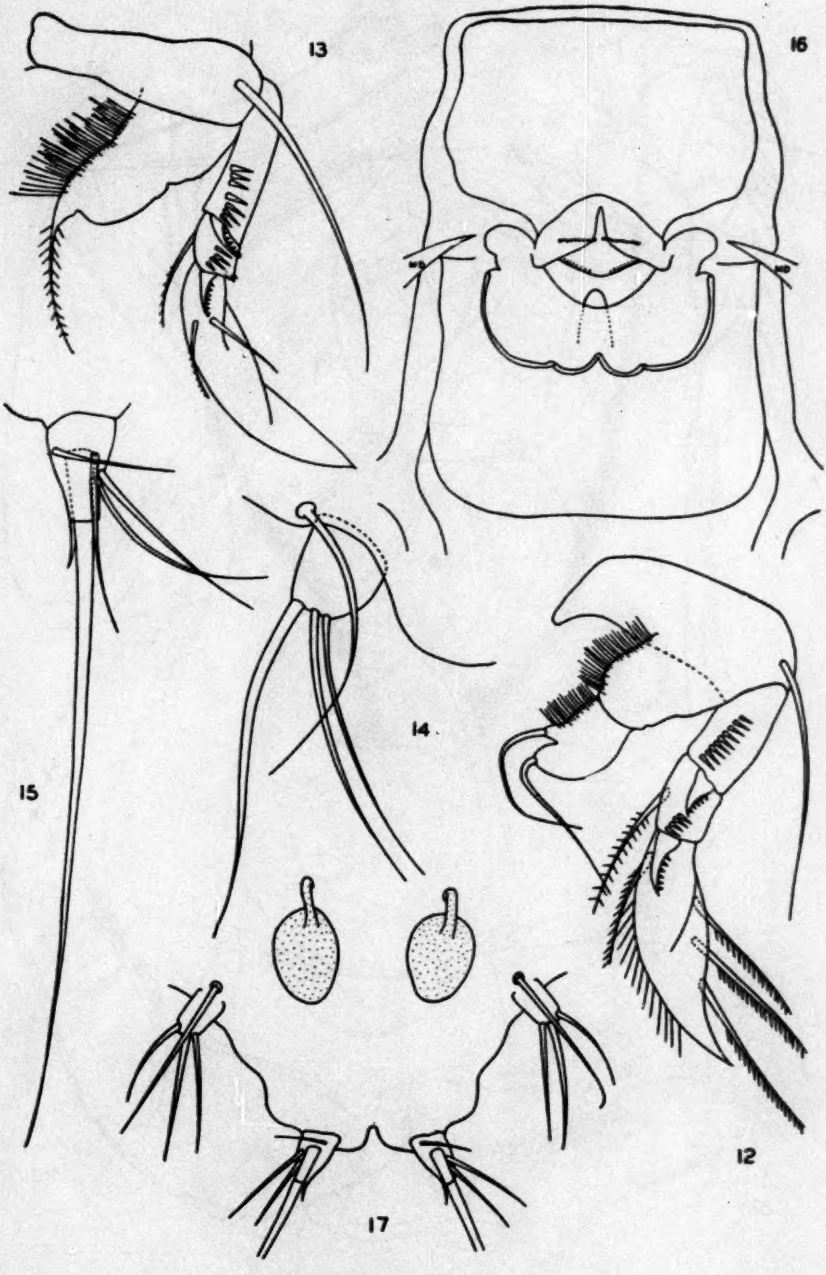


PLATE IV

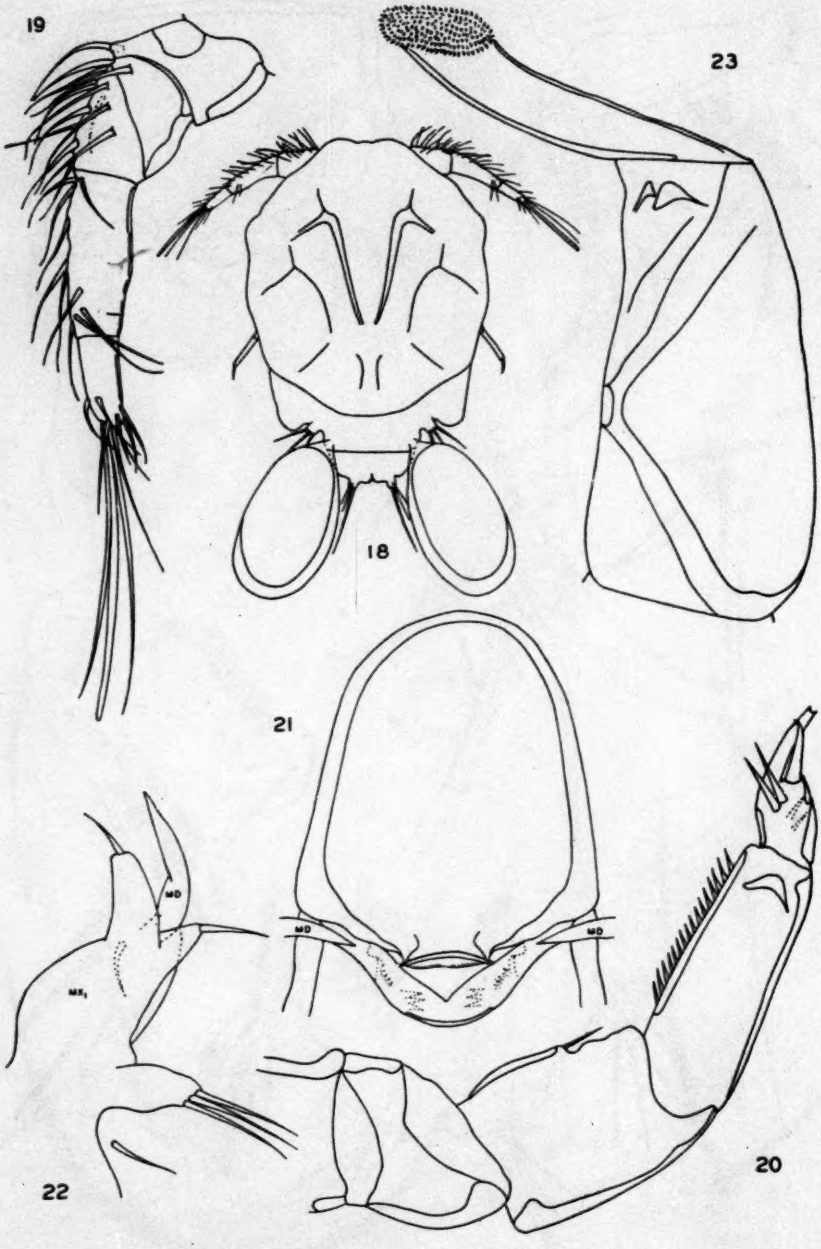


PLATE V

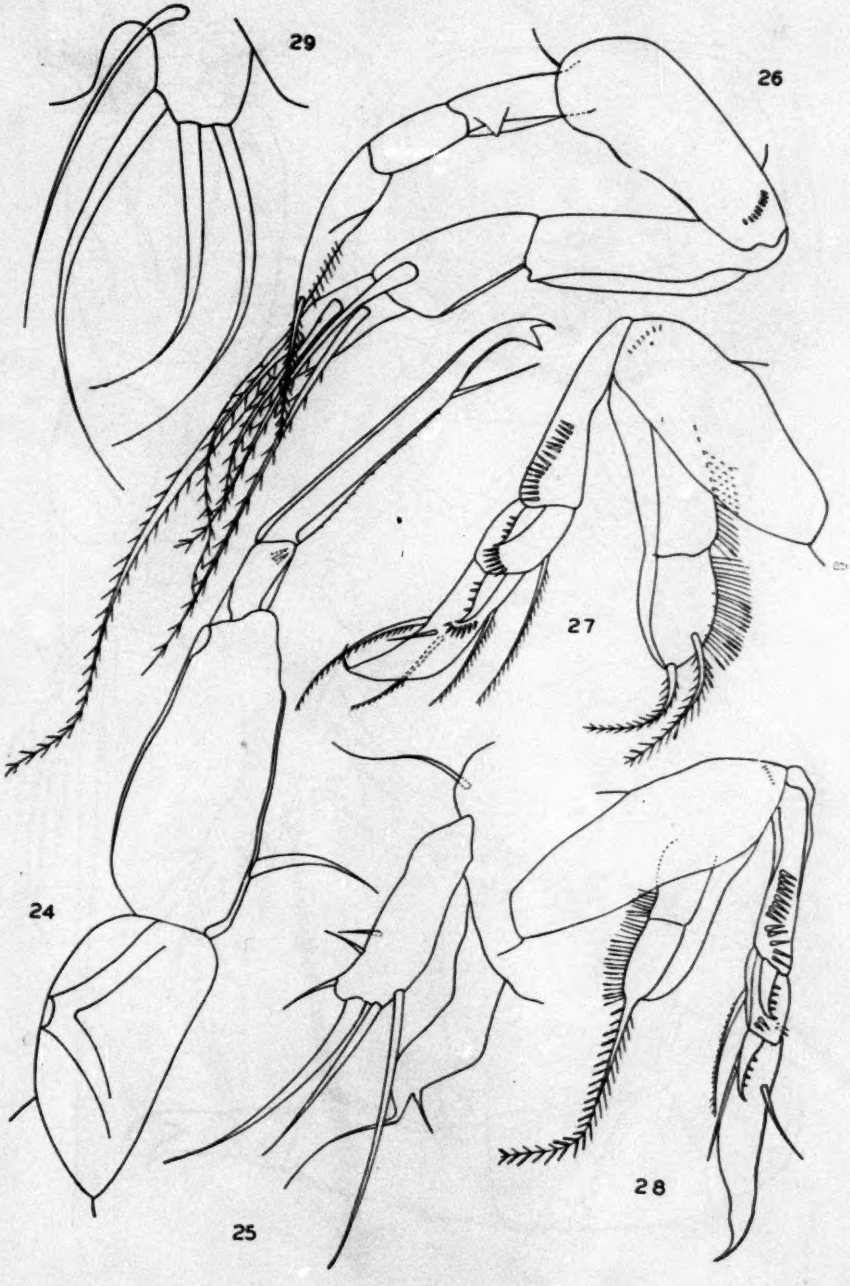


PLATE VI

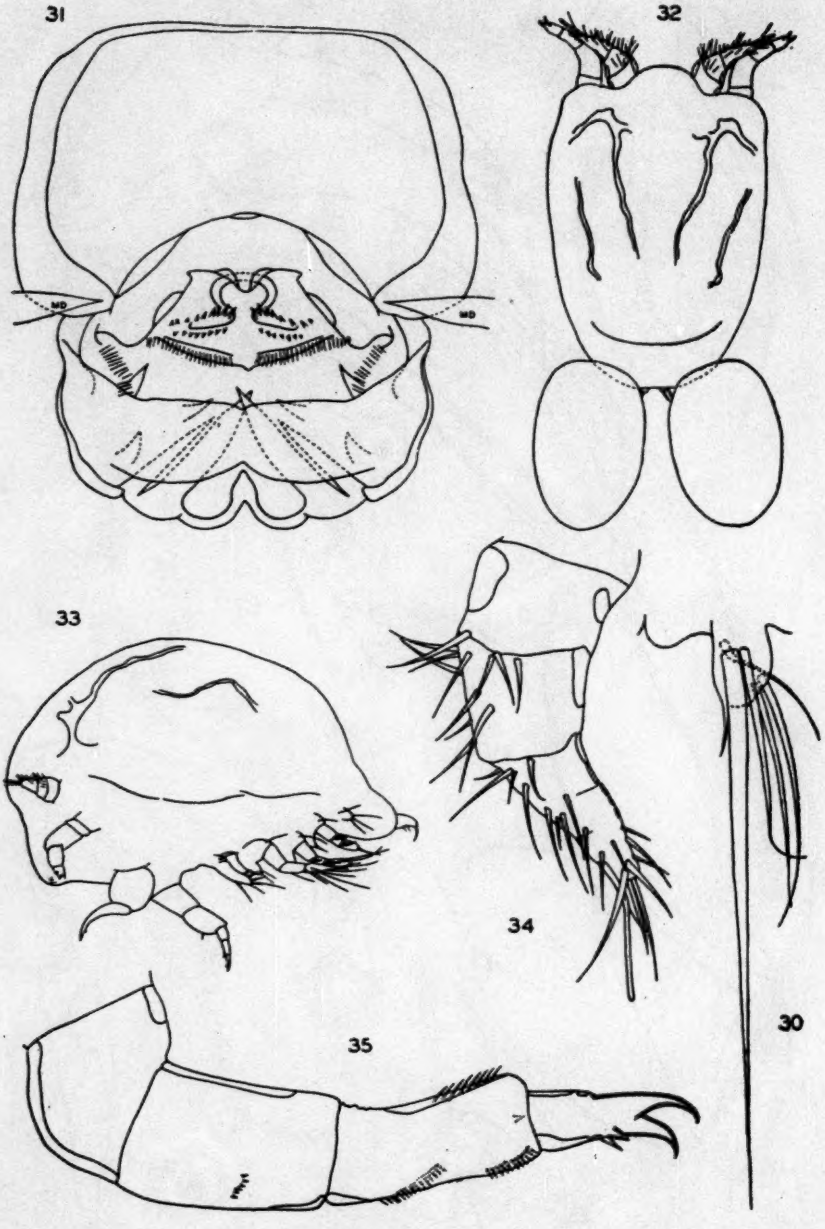


PLATE VII

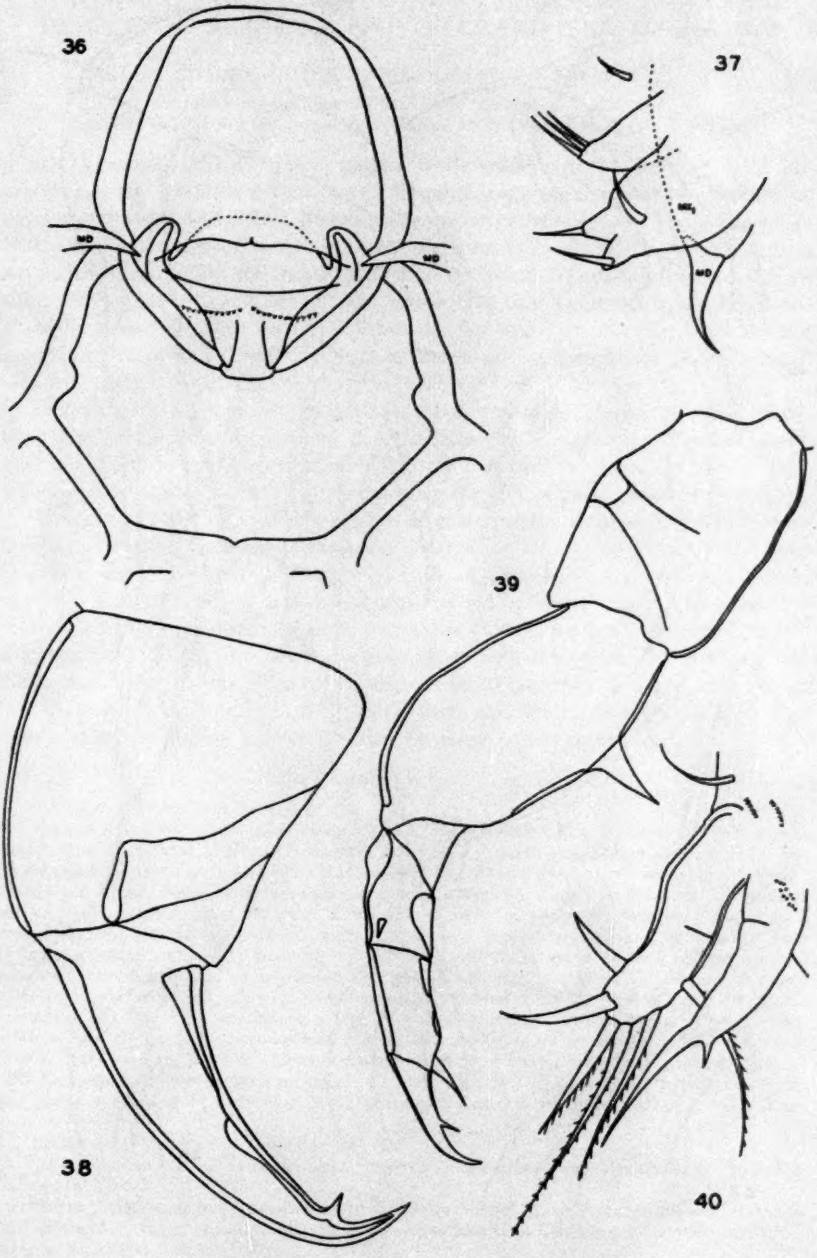
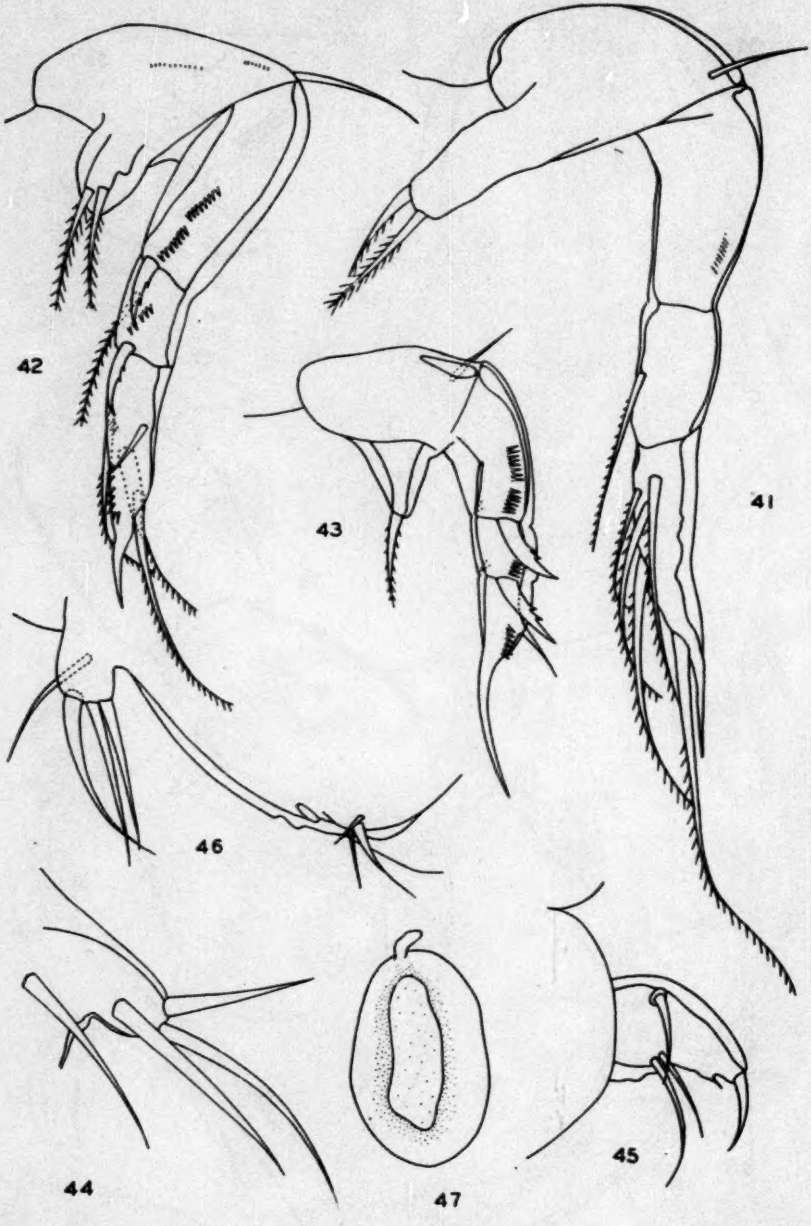


PLATE VIII



A NEW SPECIES OF *BUCEPHALOIDES* (TREMATODA: BUCEPHALIDAE) FROM A MARINE FISH OF GRAND ISLE, LOUISIANA*

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In December, 1951, the junior author, while working at the Texas A & M Research Foundation Marine Laboratory, found a single specimen of an undescribed species of *Bucephaloides* in a flounder, *Paralichthys lethostigmus* Jordan and Gilbert. This gasterostome was killed with gentle heat, fixed under cover glass pressure with Gilson's fluid and stained with alum cochineal. The senior author studied this species alive at Grand Isle in 1951. Since that time numerous examples of this *Bucephaloides* have been recovered from the flounder. It was discussed but not named or described by Sparks in a dissertation on the geographic distribution of digenetic trematodes of shallow water fishes in the Gulf of Mexico.**

The gasterostomes of Grand Isle have been studied by Hopkins (1954, 1956), who described 4 new species and listed a fifth named species as present there. Sparks (1957) listed 12 species, including the 5 above, as present at Grand Isle; 4 species were undescribed. This is a description of 1 of those undescribed species.

Melugin (1940) listed a new species of gasterostome from *Paralichthys lethostigmus* which she named *Bucephalopsis bennetti* for Dr. Harry J. Bennett. Since this form was designated by name only, with no description, its name is a *nomen nudum*. Melugin's unpublished thesis was studied by Sparks; her description and drawing fit the form discussed here. Hopkins (1956) replaced the generic name *Bucephalopsis* Nicoll, 1914, *nec* Diesing, 1855, with *Bucephaloides* Hopkins. It has seemed better to retain the trivial name *bennetti*, given by Melugin, rather than to introduce a new name. Manter (1954) listed this species as *Bucephalopsis bennetti* Melugin, 1940, but pointed out that the name was a *nomen nudum*.

Bucephaloides bennetti n. sp.

With the characters of the genus.

Body length 2 to 3 times width (length 1.09–1.72 mm, width 0.50 to 0.72 mm in specimens at hand). Widest at level of pharynx, which is 1/3 to 2/5 of body length from anterior end. Anterior sucker nearly spherical (0.14 by 0.14 and 0.18 by 0.21 in 2 specimens measured). Cephalic glands small and inconspicuous. Pharynx nearly spherical, about ½ diameter of anterior sucker. Intestine somewhat elongate, dorsal and to right of pharynx. Vitelline follicles in 2 compact groups approximately twice longer than wide, 1 on each side at level of posterior edge of anterior sucker or slightly posterior to this level, entirely anterior to level of pharynx and intestine. Ovary spherical to triangular, to right of pharynx and intestine. Testes obliquely placed, posterior to ovary; anterior testis immediately behind ovary, at level of posterior end of intestine; posterior testis median or to left of posterior end of anterior testis. Cirrus pouch about 1/3 as long as body; anterior end at level of posterior testis or slightly behind this level. Uterus convoluted, on left side of body between levels of vitellaria and cirrus pouch except for first part, which crosses body from ovary, and last part, which loops between posterior testis and cirrus pouch and expands to a broad portion adjacent to posterior end of cirrus. Eggs

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0.019 by 0.011 mm; opercular cap not apparent. Excretory bladder extending forward far beyond pharynx and intestine, almost to anterior sucker.

Type specimen: U. S. National Museum Helminthological Collection No. 38303.

DISCUSSION

Using the key of Chauhan (1943), our specimens will not key to any of the species included unless the vitelline follicles are considered to be "extending like a band along the sides of the body"; if this and some other liberal interpretations are made to fit the key, the bucephalid from the flounder can be made to key out as *Bucephaloides ovatus* (Linton, 1900). However, *B. bennetti* differs from *B. ovatus* by having a much less elongated intestine and by having the vitellaria more compact and not extending as far posterior; in *B. ovatus* the vitellaria overlap the level of the pharynx, according to Linton (1940).

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PLATE I

EXPLANATION OF FIGURES

FIGURE 1. *Bucephaloides bennetti*, ventral view. Specimen collected by S. H. Hopkins at Grand Isle, Louisiana; camera lucida drawings of the same individual while alive and after staining and mounting, combined into one. Scale line equals 0.2 mm.

FIGURE 2. *Bucephaloides bennetti*, ventral view of mounted specimen collected by A. K. Sparks at Grand Isle, Louisiana. Camera lucida drawing. Scale line equals 0.2 mm.

FIGURE 3. Eggs of *Bucephaloides bennetti*, drawn from balsam mounts with camera lucida. Scale line equals 0.02 mm.

A NEW NOTOCOTYLID TREMATODE, *UNISERIALIS GIPPYENSIS* GEN. ET SP. NOV., FROM THE MALLARD, *ANAS PLATYRHYNCHA PLATYRHYNCHA* L.

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Forty-nine trematodes were recovered from the intestinal ceca and bursae Fabricii of 3 mallards examined in November 1956 and October 1957. All were taken at Nacton Decoy, near Ipswich, Suffolk, England.

The specimens were fixed in cold formal-acetic under slight coverslip pressure and stained with aceto-carmine. Some of the specimens were subsequently unmounted and stained with picro-nigrosin to demonstrate the pattern of the ventral glands more clearly. Several specimens were sectioned transversely. These trematodes appear to represent a new genus for which the name *Uniserialis* is proposed.

Uniserialis n. g.

Diagnosis: Notocotylidae Lühe, 1909; body flattened, small, rather pointed at the anterior end but rounded posteriorly; cuticle aspinose. Ventral surface with a single median longitudinal row of sessile glands. Oral sucker terminal, pharynx absent; esophagus short leading to intestinal bifurcation, ceca simple ending blindly near posterior extremity. Ventral sucker absent. Common genital pore ventral, median, anterior to intestinal bifurcation, near oral sucker. Two lobed testes, posterior and extracecal in position; external vesicula seminalis well developed. Cirrus sac elongate with internal vesicula seminalis; cirrus unarmed. Ovary median, between the testes, immediately posterior to Mehlis' gland; receptaculum absent. Uterus with ascending limb only, forming intracecal transverse slings. Metraterm long, with thickened walls and opening at the genital pore. Vitellaria follicular, in 2 lateral extracecal bands, running forward from anterior border of testes. Excretory pore dorsal and posterior, receiving the 2 main excretory vessels. Eggs numerous small and operculate with long polar filaments. Adults in intestinal ceca and bursa Fabricius of birds. Genotype: *U. gippyensis* n. sp.

Uniserialis gippyensis n. sp.

Specific description: With characters of the genus. Body small, 1.37–2.54 long and 0.63–1.27 in maximum width at level of transverse uterine coils. Ventral surface with single median longitudinal row of 5 large sessile glands which are coral pink and conspicuous in unfixed material. Anterior gland situated in posterior part of anterior third of body overlying base of cirrus sac; second gland immediately behind first. Third or median gland lying just inside posterior half of body, widely separate from second and fourth glands. Latter situated in posterior third of body, immediately anterior to fifth or posterior gland which is ventral to ovary. Anterior and posterior glands of series noticeably smaller than other glands; median gland largest. In all specimens examined all glands are more or less protruded and have transverse slit-like openings. Oral sucker terminal, globular or sub-globular, measuring 0.12–0.17 × 0.12–0.18; mouth directed forwards. Short slender esophagus up to 0.13 in length, leads to bifurcation of gut. Intestinal ceca extend to within 0.14 of the posterior end of body. Ceca diverge in region of transverse uterine coils, converging to pass between testes; in post-testicular region ceca slightly distended and more widely separate. Testes longer than wide, extra-cecal and lying in posterior region of body. Right testis 0.19–0.51 × 0.17–0.28, and left 0.19–0.42 × 0.15–0.36. Both testes lobed on outer margins; inner margins with less pronounced lobation. External vesicula seminalis well developed and dorsal to uterus. Distal portion of external vesicula seminalis lying in longitudinal axis of body but anteriorly forming 2 or 3 large loops before passing into cirrus sac. Cirrus without spines or papillae and lying in an elongate, clearly demarcated cirrus sac which measures 0.44–0.80 × 0.07–0.14. Base of cirrus sac situated in second third of body. Cirrus sac also containing internal vesicula seminalis and pars prostatica. Ovary slightly lobed 0.11–0.24 × 0.10–0.24 lying in posterior region of body between testes. Mehlis' gland just an-

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terior to ovary in position and measuring 0.06–0.12 × 0.08–0.15. No receptaculum seminis. Uterus with ascending limb and prominent transverse slings which are entirely intracecal. Total number of uterine slings varying between 8 and 13, number anterior to vitellaria between 3 and 5. Metraterm nearly as long as cirrus sac; when full of eggs extremely difficult to see, but when empty or containing only a few eggs thickened walls are visible. Metraterm and cirrus sac opening at common genital pore which is slit-like and situated, anterior to the intestinal bifurcation, near the oral sucker and only 0.09–0.19 from anterior margin of body. Vitellaria composed of distinct, irregular follicles measuring 0.02–0.03 × 0.03–0.06. Vitelline follicles arranged in longitudinal extracecal bands on either side of body, confined to posterior half of body and with posterior follicles overlying anterior border of testes. Vitelline ducts passing between testes and posterior uterine slings to meet in the midline, dorsal to Mehlis' gland. Excretory pore dorsal and posterior lying mid-way between posterior margin of body and ovary. Two excretory canals run from esophageal region, parallel anteriorly with ceca, to small excretory bladder lying ventral to excretory pore. Eggs thin-shelled, colorless and operculate, 18–20 × 11–13 microns and bearing polar filaments up to 0.113 long.

Host: Anas platyrhyncha platyrhyncha L.

Locations: Intestinal ceca and bursa Fabricii.

Locality: Nacton Decoy, near Ipswich, Suffolk, England.

Co-type: To be deposited in the collection of the British Museum (Natural History).

DISCUSSION

This new genus shows affinities with other genera of the Notocotylidae Lühe, 1909, especially those of the sub family Notocotylinae Kossak, 1911, which includes *Notocotylus* Diesing, 1839, *Catatropis* Odhner, 1905, *Paramonostomum* Lühe, 1909, *Quinqueserialis* Skvorzov, 1934, *Hofmonostomum* Harwood, 1939 and *Tristriata* Belopolskaia, 1953.

Diesing (1839) erected the genus *Notocotylus* for specimens of "typical" notocotylid internal anatomy and with 3 longitudinal rows of ventral glands. As Dubois (1951) pointed out there was still considerable confusion over the genus until Odhner (1905) erected the genus *Catatropis* for those notocotylids with a midventral keel, on either side of which is a row of glands. Later workers, Noble (1933), Johnston (1928) and Skrjabin (1953), disregarded the presence of a ventral keel as a generic feature in *Catatropis* and attempted the separation of *Catatropis* and *Notocotylus* using differences in the evaginability of the ventral glands and in the ratio of the measurements of the cirrus sac and metraterm. The genus *Paramonostomum* was erected by Lühe (1909) for those species which have no ventral glands or ridges. As noted by Harwood (1939), it is probable that several species described as *Paramonostomum* are actually *Catatropis*. The genus *Quinqueserialis* Skvorzov, 1934 is based on the presence of 5 rows of ventral glands. *Hofmonostomum* Harwood, 1939 has weak glands in the median row which open onto a keel-like ridge; there are no lateral glands and the vitellaria extend posterior to the testes. The most recently described genus, *Tristriata* Belopolskaia, 1953 has 3 large ventral ridges.

Uniserialis gippyensis bears a superficial resemblance to *Notocotylus skrjabini* Ablassov, 1953, but differs from this species not only in the pattern but also in the number of the ventral glands, the position of the common genital pore, and the relative measurements of the cirrus-sac and metraterm.

While the validity of genera based on the differences of the ventral glands and/or ridges has been questioned by Baylis (1928), Duthoit (1931) and Harwood (1939), there does not appear to be at present an alternative character which can be used for the separation of the genera mentioned above. Szidat & Szidat (1933) and Szidat (1935) showed that in the genus *Notocotylus* the *pattern* of the ventral

glands is more or less constant for any given species. Both Harwood (1939) and Skrjabin (1953) rely on the pattern of the glands and ridges in their keys for the identification of the genera of the sub-family Notocotylinae.

The present material is unlike any of the above genera as it has a single row of clearly separate, sessile glands. Therefore a new genus *Uniserialis* is erected with *U. gippyensis* as type species.

SUMMARY

A new trematode, *Uniserialis gippyensis* n. g., n. sp., from the Mallard, *Anas platyrhyncha platyrhyncha* L. is described.

The separation of the genera of the sub-family Notocotylinae Kossak, 1911, is discussed.

ACKNOWLEDGMENTS

I wish to thank Professor B. G. Peters under whose supervision this work was carried out, and also Mr. S. Prudhoe of the British Museum (Natural History) for his interest and suggestions. I am indebted to the Suffolk Naturalists Society for a grant towards the cost of purchasing ducks and to Mr. A. E. Baker, Decoy Keeper, at Nacton.

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EXPLANATION OF PLATE

FIGURE 1. *Uniserialis gippyensis* n. g., n. sp.

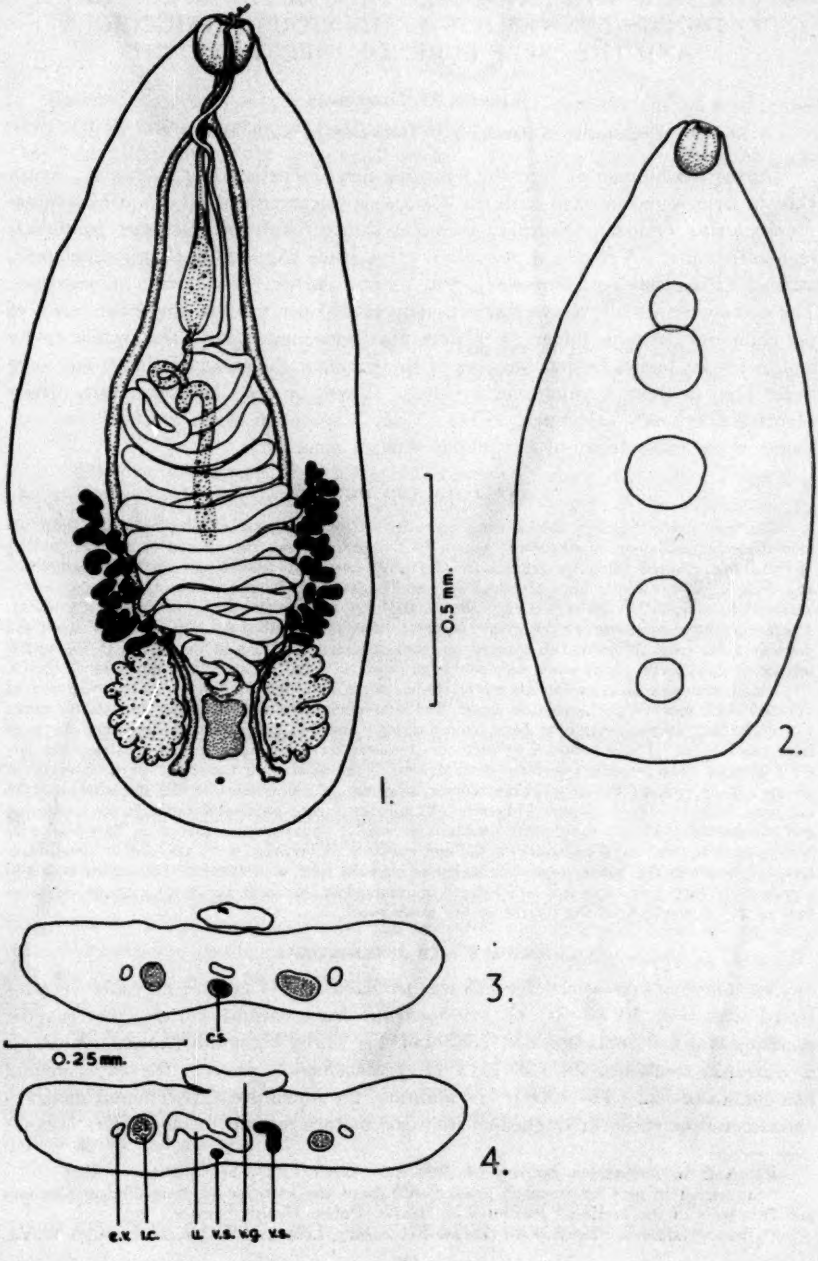
FIGURE 2. Pattern of the ventral glands.

FIGURE 3. Transverse section through the anterior part of the second ventral gland.

FIGURE 4. Transverse section through the median part of the second ventral gland.

Abbreviations used: c.s.—cirrus sac; e.v.—excretory vessel; i.c.—intestinal cecum; u.—uterus; v.g.—ventral gland; v.s.—vesicula seminalis.

PLATE I



STUDIES ON THE LIFE-CYCLE OF *ORNITHODIPLOSTOMUM PTYCHOCHEILUS* (FAUST) (TREMATODA: STRIGEOIDEA) AND THE "SELF CURE" OF INFECTED FISH*

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During the summer of 1956 the following fish of Turtle River (Arvilla), North Dakota were found infected with the *Neascus* metacercaria of *Ornithodiplostomum ptychocheilus* (Faust): Northern common shiner (*Notropis cornutus frontalis*), bigmouth shiner (*Notropis d. dorsalis*), creek chub (*Semotilus a. atromaculatus*), fathead (*Pimephales p. promelas*), and johnny darter (*Boleosoma n. nigrum*). The cysts were usually very numerous (up to 400 per fish) in the mesenteries of the chub and common shiner; they were also very numerous in the cranial cavity and in the fat bodies behind the eyes of the common shiner and fathead, but were never seen in these locations in the chub. These unusual locations were briefly reported previously (Hoffman, 1954). Only 1 specimen of *O. ptychocheilus* was found in the mesenteries of the johnny darters examined.

MATERIALS AND METHODS

Thirteen newly hatched chicks were force-fed 100–450 metacercariae which had been removed mechanically, or by 10-minute pepsin-HCl digestion from the viscera or cranial cavities of the above infected fish; An average of 23 (0–89) ovigerous adults was recovered per chick at 2–5 days. These adults were identical with adults described from mergansers (*Mergus americanus, M. serrator, Lophodytes cucullatus*) and the old squaw duck (*Harelda hyemalis*). There was no morphological difference in the adults recovered from chicks which were fed metacercariae from different fish species, or from different locations in the fish. As is characteristic of this species, there were very few eggs *in utero*, usually 1 or 2, and never more than 6.

Small numbers of eggs for life cycle studies were collected in 3 ways: (1) collection of infected chick feces in dechlorinated water, and subsequent straining, washing and sedimenting; (2) collecting worms containing eggs from autopsy; and (3) mechanically removing the eggs from the worms. Those obtained by method (1) were incubated with forced aeration; the latter 2 groups were incubated in 5-cm petri dishes. Free-swimming miracidia were observed in group (3) at 9 days; the eggs in the worms of group (2) developed to the miracidium stage but none hatched. Both groups (1) and (3), however, were successful methods for collecting and incubating eggs for subsequent infection of snails. Snails were placed in half-pint milk bottles after several days' exposure to the egg cultures. Cercariae were allowed to accumulate for 6–12 hours in the bottles and then added to about a liter of aerated dechlorinated water in a gallon jar with 1 or more fish which were maintained in the same jar at an average temperature of 24° C throughout the course of the experiment.

RESULTS AND OBSERVATIONS

No source of trematode-free fish was available but 11 control fatheads were infected with only 10 (0–40) *O. ptychocheilus* from natural sources, whereas the experimental fish contained 832 (387–1441). Three bigmouth shiners (*Notropis d. dorsalis*) contained 70 (38–131) *O. ptychocheilus*, whereas the experimental fish contained 722 (387–1368). In addition, the immature experimental metacercariae could be easily distinguished from the mature natural metacercariae because

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the experiments were done in late fall (October 30–November 2) and the natural metacercariae were mature by the time the earliest experimental fish were examined.

All measurements given below are in microns unless otherwise stated.

The Miracidium

Because of a scanty supply of eggs only 3 miracidia were studied. These moved very rapidly in the usual spiral fashion of strigeoid miracidia except that sometimes they swam intermittently in very small circles. The following description is based on 2 specimens only: Size 127–171 by 46–49; eye spots 8–10 by 5 dorsally, and very close together with concave sides facing each other and containing what appear to be 2 lenses; cilia *ca* 10 long; a rather large oval structure, 30 in diameter, containing cells with nuclei 5 in diameter, just posterior to eye spots.

The Sporocyst

Thirty-six *Physa anatina*, 27 *Stagnicola palustris*, and 20 *Helisoma anceps*, all laboratory-reared, were exposed to cultures prepared as described above; of these 5 *Physa anatina* became infected and cercariae first emerged at 45 days.

Mother sporocyst:

One mother sporocyst 2.8 × 0.1 mm was removed from a snail liver; 1 end was slightly invaginable and may have contained the birth pore. No distinguishing morphological features were noted.

Daughter sporocyst:

These were also in the liver. Three were 232–490 by 40–54. No ear-like structures were seen and the birth pore is apparently at the anterior end, which is slightly invaginable; germ balls were *ca* 24 in diameter.

The Cercaria

The cercaria of *O. ptychocheilus* is of the longifurcate monostome type. No acetabulum, pharynx, esophagus or ceca can be ascertained. The penetration organ (oral sucker) is oval, elongate and *ca* 50 by 17 alive. The anterior tip is usually inverted. Small scattered spines, *ca* 1.5 long, cover the anterior surface of the cercaria for *ca* ½ the length of the penetration organ; those of the area corresponding to the anterior end of the penetration organ are shorter. Posterior to this crown of spines are 8 single transverse rows of small spines *ca* 1 long which can be seen with phase only; the terminal row is at the level of the first pair of penetration glands. One pair of laterally projecting flagellets, 10 in length, emerges from the body between the posterior pair of penetration glands and the posterior end of the body.

There are 6 pairs of flame cells in the body of the cercaria plus 2 in the tail-stem. Two pairs are located postero-laterally to the penetration organ, 2 centrally, and 2 posterior. Only the 2 main lateral excretory ducts of the body and the main tail-stem tubules could be discerned. The excretory bladder is bipartite with a small "island of Cort".

A pair of simple eye spots, *ca* 3 to 4 in diameter, is located just posterior to the middle of the body, but cannot always be demonstrated. Three pairs of penetration glands, *ca* 10 in diameter, lie posterior to the eye spots but do not reach

the posterior end of the body; ducts lead anteriorly from them through the penetration organ, in which they are enlarged, and open in small pores lateral to the oral opening. Between the eye spots is a body of cells which are *ca* 2 in diameter. Between the penetration glands are 2 tandem bodies composed of cells *ca* 4 in diameter. Posterior to the last pair of glands is a larger body of cells which is probably the genital Anlage.

The body is 86/100 as long as the tail-stem which is slightly narrower. Most of the cercariae have 5 pairs of caudal bodies, but in some newly emerged cercariae there are 6 pairs which extend almost to the furcae; these bodies disappear (become used up?) at about 12 hours after the emergence of the cercaria. There are 2 pairs of flame cells in the tail-stem, 1 anterior and 1 near the posterior end. The excretory duct from the excretory bladder passes through the center of the tail-stem and bifurcates at the origin of the furcae; each duct proceeds to the proximal anterior surface of the furca where it empties through a minute pore about 2/3 the distance from the furcal base. The surface of the tail-stem bears about 60 annulations which correspond to underlying cells. There are 8 to 10 flagellets *ca* 10 in length on each side of the tail stem. Very small flagellets, *ca* 1 long, are present on the anterior edge of the furcae. A very small fin-fold extends from about midway on the anterior edge of the furca and around the end to about midway of the posterior edge.

Measurements of 10 specimens fixed in hot formalin: Body 169 (144–196) by 31.8 (26–39), tail stem 196 (162–220) by 27 (24–34), furcae 177 (159–196) by 13 (12–14) dorsal aspect, greatest lateral width of furcae 19, penetration organ 66 by 19.

This cercaria is almost identical with the cercaria of *Posthodiplostomum minimum centrarchi* as described by Miller (1954). However, the following differences are noted: *Cercaria O. ptychocheilus:* (1) is smaller—the body is 12% smaller, tail-stem 21% smaller, furcae 19% smaller; (2) has 8 rows of body spines instead of 10; (3) has 6 pairs of body flame cells instead of 8; (4) has a slightly longer tail-stem in proportion to the body-length; (5) has an anterior area of very short spines instead of all anterior spines of equal size; (6) sometimes has 6 pairs of caudal bodies instead of 5. The writer realizes that some of these features may be variable and are not generic differences. It seems apparent that the 2 cercariae are very closely related; perhaps the 2 worms should be placed in the same genus.

Fish Infection and Metacercarial Development

All of the following fish became infected: 11 fatheads (*Pimephales p. promelas*), 9 central bigmouth shiners (*Notropis d. dorsalis*), and 1 creek chub (*Semotilus d. dorsalis*). However, neither of 2 johnny darters (*Boleosoma n. nigrum*) or 1 stickleback (*Eucalia inconstans*) became infected. Surprisingly, 1 johnny darter from Turtle River was found with 1 natural *O. ptychocheilus* metacercaria. The experimental fish became infected with large numbers and the "crowding effect" probably retarded the development of the metacercariae as well as the development of the cyst of parasite origin in some instances. The development was very similar to that of *Posthodiplostomum minimum centrarchi* as reported by Hoffman (1958) and Miller (1954).

Development of the Metacercaria and Parasitic Cyst

7 days—Many metacercariae are found in the mesenteries, cranium, fat bodies behind the eyes, and in the vitreous chambers. They are very actitve and plastic, and measure 313 to 732 long by 188 to 260 wide. The penetration ducts are still present, the holdfast can be seen as a slight invagination, but the ventral sucker can not yet be ascertained. At this stage the metacercariae can not be differentiated from larvae of *Posthodiplostomum minimum* of similar age.

10 days—No evidence of cyst of parasite origin yet, but the metacercaria appears nearly mature.

17 days—The secreting of the cyst of parasitic origin is in progress. Some were left overnight in saline and the parasite cyst separated from some of them as reported for *Posthodiplostomum* by Hoffman (1958).

20 days—Many parasites are enclosed in "swollen" recently secreted parasite cysts. The metacercaria itself has a swollen appearance and cannot be distinguished from *Posthodiplostomum* at this time; in fact, the writer could not identify the parasite certainly until after this stage of development.

24 days—In fish with a moderate number of parasites the cysts and the parasites were fully formed. However, many of the metacercariae in very heavily parasitized fish were retarded in development and appeared like the 10–17-day stage above.

55 days—Of 2 heavily infected fish 1 had a few immature metacercariae, the rest being mature, and in the other, 20% were immature, presumably because of the "crowding effect." A few dead metacercariae were observed.

107 days—In 1 extremely heavily infected fish all of the 1368 metacercariae appeared mature and normal except for a few dead ones, and a few which were about 2/3 as large as the rest.

The Host Cyst

The cysts can be easily removed from adjacent tissue, and the larvae easily liberated by tearing the cyst with dissecting needles. Experimental cysts, 20 to 68 days old, were studied in histologic sections stained with hematoxylin and phloxine, and Mallory's triple connective tissue stain. The cyst of parasite origin stains blue with Mallory's and is *ca* 1.6 microns thick. At 20 days the host cyst is *ca* 3 microns thick, and is composed of closely packed connective tissue which stains red with Mallory's stain at this time; the nuclei are oval and *ca* 16 × 13 microns in diameter.

At 68 days the nuclei can no longer be seen and the host cyst stains purplish with Mallory's stain. The cysts from the eye orbit, viscera, brain, and muscle are essentially the same.

Effect on the Fish and the "Self-Cure"

It is not uncommon to find large numbers of metacercariae in fish and often there is no evidence of deleterious effect on them. Fischthal (1953) has summarized the effects of parasitism on the fish host. Sillman (1957) observed no deleterious effect of *Posthodiplostomum minimum centrarchi* on a sunfish in an aquarium although the fish's kidney and liver were riddled with parasites. It is difficult to understand how fish can withstand extremely heavy infections and remain

apparently healthy. Hoffman (1958) reported a peculiar means by which excessive numbers of larval *Posthodiplostomum m. minimum* are eliminated by minnows—a bursting of the body wall, allowing some of the parasites to fall out followed by closure of the wound. In the present study this rupture was repeated with *O. ptychocheilus*. The 19 fish (*P. p. promelas, N. d. dorsalis, S. a. atromaculatus*) were exposed to relatively large numbers of uncounted cercariae and of these 19, 12 were examined carefully for evidence of lacerations at 31 to 107 days post-infection; 8 showed evidence of having burst, releasing parasites, followed by complete healing (Fig. 1). Histopathologic examination of the affected area showed simple scars completely through the body wall. The fish were not examined for an acumulation of tissue fluid (ascites) in the body cavity and there is a possibility that it might have contributed to this phenomenon.

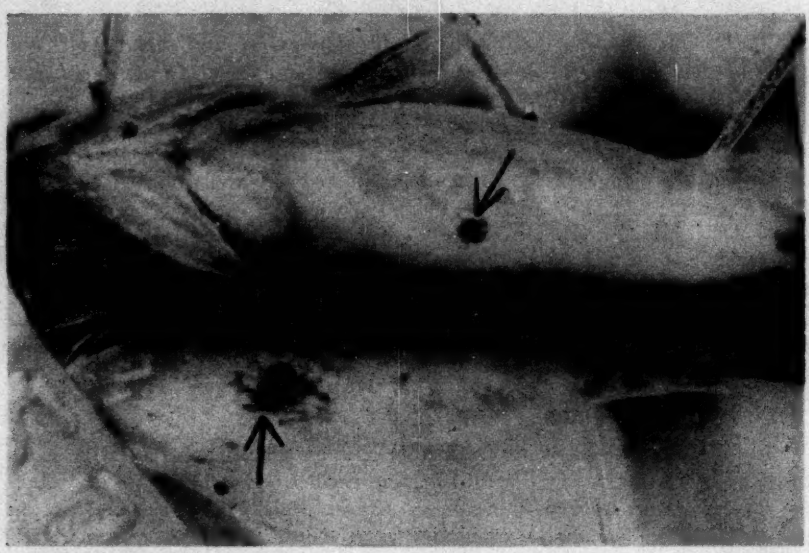

FIGURE 1. Two *Pimephales p. promelas* 40 days post-infection with a large number of cercariae of *Ornithodiplostomum ptychocheilus*. Note the large dark scars where the body wall had burst and healed over.

There was considerable hyperemia at the base of the fins of several of the infected fish at 1 to 24 days post-infection; in 1 instance several spots of hyperemia on the body were also seen.

In moderate natural infections the metacercariae were almost always in 2 locations, the body cavity and on the brain. In the heavy experimental infections, however, the metacercariae were found in 3 additional locations: the eye orbit, the vitreous chamber of the eye (occasionally subcorneal), and in the musculature. The larvae from 5 experimentally infected fish were counted with the following results: (1) total, average 832 (387–1441) (2) body cavity, 89.2% of total average (3) eye orbits, 4.5% (4) eyes, 4% (5) brain, 1.8%, (6) musculature, 0.5%. During the examination of histologic sections of the brain several larvae were noted in the brain tissue itself; most, however, were on the surface of the brain.

Except for the afore-mentioned hyperemia and the thin connective tissue host cyst, there appears to be little host reaction; when numerous, some of the cysts on the surface of the brain are sunken into the brain tissue, presumably because of resorption, and the meninges are intact, although thinner, beneath the cyst. In very heavy infections the meninges become considerably thicker and very vascular adjacent to the cysts. The cysts in the brain tissue itself are essentially identical with those on the surface. No unusual physical symptoms were noticed except that 1 experimental fish became greatly emaciated.

SUMMARY

The life cycle of *Ornithodiplostomum ptychocheilus* is described. Adults were obtained by force-feeding baby chicks with metacercariae from the mesenteries and brain of the shiner (*Notropis cornutus frontalis*) and fathead (*Pimephales p. promelas*) and mesenteries of the creek chub (*Semotilus a. atromaculatus*). Snails (*Physa anatina*) were infected with the resulting miracidia, and the sporocysts and cercariae are described. Fish (*Pimephales p. promelas, Notropis d. dorsalis, Semotilus a. atromaculatus*) were experimentally infected and the development of the metacercaria and cyst is described. A bursting of the body wall of the fish, resulting in release of metacercariae ("self-cure") is described. The histopathology of the infected fish is briefly described.

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COMPARISON OF NUTRITIVE RESERVES IN MALES OF AUTOGENOUS AND ANAUTOGENOUS POPULATIONS OF *CULEX PIPIENS*

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The autogenous form of *Culex pipiens* L., which for convenience is called *molestus*, is characterized by the ability of the females to lay eggs without having taken a blood meal. Roubaud and Toumanoff (1930) estimated that the reserves carried over from the larva to the pupa were 3 times greater in *molestus* than in *pipiens*. Clements (1956) noted that the fat body in the larva of *C. pipiens* was less extensive than in *molestus*, but nevertheless contains enough reserves to permit at least some eggs to develop. Twohy and Rozeboom (1957) compared quantitatively the fat, glycogen, and nitrogen composition of pupae and adult females of *molestus* and *pipiens* reared under comparable conditions, and found significantly greater amounts of these materials in *molestus*. However, the authors agree with Clements' conclusion that some hormone must be involved in stimulating ovarian growth.

If the mechanism whereby *molestus* females are assured of producing autogenous eggs is primarily an accumulation of excessive food reserves during the larval stage, a comparable accumulation of reserves might not occur in the males. The purpose of this study was to make quantitative comparisons of fat, glycogen and nitrogen in male pupae and adults of *molestus* and *pipiens* to determine if the accumulation of greater food reserves is only a characteristic of the autogenous females or a characteristic of both sexes of *molestus*.

MATERIALS AND METHODS

The pupae were taken from the same rearing pans that furnished the females of our earlier studies. Larvae of *molestus* and *pipiens* were reared in uncrowded pans, placed alternately on tables in our insectary, and fed adequate amounts of the same food. Samples were taken of pupae representing 2 age groups: 0 to 24 hours and 48 to 72 hours (mean ages of 12 and 60 hours). The ages of the adult samples were 3–4 days, 5–6 days, and (in the case of *molestus*) 7–8 days from the time of pupation (mean ages of 84, 132 and 180 hours). No *pipiens* adults lived for 7–8 days.

The procedures followed in collection and storage of specimens, and in most of the analyses, were the same as those described by Twohy and Rozeboom (1957), except that nitrogen determinations were made according to the Nessler method. Dry weights and lipids were determined with samples of 30 to 103 mosquitoes, glycogen with samples of 4–8 mosquitoes, and nitrogen on samples of 1 or 2 specimens. Values for glycogen and nitrogen represent averages of 2 analyses.

RESULTS

Table I shows the values obtained for the various components. Because of the obvious differences in sizes of the mosquitoes in the two experiments, the results of each experiment are presented separately. These differences perhaps reflect differences in temperature or other environmental influences, which we were unable

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TABLE I. *Comparison of dry weight, ether soluble lipids, glycogen, and nitrogen in males of* C. pipiens *and* molestus *in micrograms per mosquito.*

Exp. No.	Stage	Age[1] (hrs.)	Dry weight mol.	Dry weight pip.	Lipids mol.	Lipids pip.	Glycogen mol.	Glycogen pip.	Nitrogen mol.	Nitrogen pip.
2[2]	P	12	631	498	156	91.1	67.8	38.6	50.2	47.8
	P	60	615	454	122	63.5	44.3	19.2	56.0	45.8
	A	84	500	363	95.8	51.2	56.4	21.2	47.7[3]	34.6
	A	132	401	284	61.7	7.87	35.7	5.2	35.2	31.9
	A	180	319		47.2		9.6		34.0	
3	P	12	835	687	253	178	72.7	61.5	64.0	47.2
	P	60	729	577	219	167	57.2	38.8	59.3	56.5
	A	84	618	500	192	132	70.2	52.4	45.4	46.4
	A	132	525	406	133	75	54.1	13.9	49.9	42.6
	A	180	430		84		25.0		45.8	

[1] Mean age from time of pupation.
[2] Males in Exp. 1 were not collected. Exp. 1 was described by Twohy and Rozeboom (1957).
[3] Based on one determination only.

to duplicate from experiment to experiment. This might be a serious source of error in interpreting these results as valid for natural populations of *pipiens* and *molestus* in all environmental conditions. However, we consider that the results are valid insofar as they demonstrate consistent differences between the developmental stages of *molestus* and *pipiens* within each experiment and under conditions similar to those of our insectary.

TABLE II. *Comparison of differences in components between* molestus *and* pipiens *in micrograms per mosquito.*

Component	Exp. No.	$\bar{x}$ (Mean difference)	$SE_{\bar{x}}$	P
Dry weight	2	137	9.1	< .01
	3	135	9.2	< .01
Lipids	2	55.4	4.3	< .01
	3	61.5	4.9	< .01
Glycogen	2	30.0	2.1	< .01
	3	21.9	6.3	< .05
Nitrogen	2	7.9	1.8	> 0.1
	3	6.5	3.6	> 0.1

By treating each set of determinations of *molestus* and *pipiens* as paired observations (excluding the 180-hour age group) one can estimate the significance of these differences by the mean difference, standard error of the difference, and P as determined by the t test. These are summarized in Table II. It will be noted that dry weights, lipids, and glycogen are significantly greater in *molestus*, but that there is not a significant difference in nitrogen.

In Table III, the components are expressed as percentages of the dry weight. Inconsistencies between experiments are probably due to the fact that the various

TABLE III. *Carcass composition of* molestus *and* pipiens *males, given as percentages of dry weight.*

	Age (hrs.)	Exp. No.	Lipids mol.	Lipids pip.	Glycogen mol.	Glycogen pip.	"Proteins"[4] mol.	"Proteins"[4] pip.
P	12	2	24.7	18.3	10.7	7.75	49.7	60.0
		3	30.3	25.9	8.71	8.95	47.9	42.9
P	60	2	19.8	14.0	7.20	4.23	56.9	63.1
		3	30.0	28.9	7.85	6.72	50.8	61.3
A	84	2	19.2	14.1	11.3	5.84	59.6	59.6
		3	31.1	26.4	11.4	10.5	45.9	58.0
A	132	2	15.4	2.9	8.90	1.83	54.9	70.2
		3	25.3	18.5	10.3	3.42	59.4	65.6

[4] "Proteins" estimated by multiplying nitrogen values by the factor 6.25 (Hawk, Oser, and Summerson, 1947).

measurements were obtained from different individuals, but expressed in the table as percentages of an average dry weight. The table shows that about 1/5 to 1/4 of the body consisted of lipids, about 1/10 was glycogen, and 1/2 or more in proteins. It shows also that *molestus* males contained somewhat higher percentages of lipids and glycogen than did *pipiens* males, and that *pipiens* contained higher percentages of nitrogenous compounds.

DISCUSSION

The results obtained with male *molestus* and *pipiens* are similar to those we observed for the females. The former is a larger mosquito, and contains larger total amounts of nutritive reserves. In the females we had observed somewhat larger differences in the percentage values for lipids and glycogen between *molestus* and *pipiens*. Thus we concluded that, as compared to total dry weight, the average *pipiens* female contained 2/3 as much lipid, and 1/2 as much glycogen as did *molestus*. Table III shows that in the males the differences in percentage values were not quite so large. However, if one applies a t test to the paired values for lipids and glycogen in Table III, the differences will be seen to be significant at the 1% level.

We would interpret our results, therefore, as a demonstration that no essential difference exists between the males and females, respectively, of *molestus* and *pipiens* in accumulation and retention of food reserves. We believe it is worthwhile to establish this point. The fact that both males and females of *molestus* accumulate larger reserves suggests to us that this is a physiological function of value in autogeny, but not the mechanism involved in initiating actual autogenous egg formation.

SUMMARY AND CONCLUSION

Comparison in male *C. pipiens* and *molestus* of total dry weight, lipids, glycogen, and nitrogen showed that the latter is a larger mosquito with significantly greater reserves of nutritive material. In the female *molestus*, part of these reserves are utilized in the formation of autogenous eggs. The present study suggests that the accumulation of these reserves is a physiological function shared by both females and males, and is independent of the mechanism involved in autogenous egg development.

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CESTODES OF MAMMALS FROM THE GREAT SALT LAKE DESERT REGION OF UTAH

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During the summers of 1953 and 1954, an extensive study was made of the endoparasites inhabiting the mammalian species residing in the region south and west of the Great Salt Lake. The present report constitutes the second part of the study; the first, on the nematodes was recently published (Grundmann, 1957). Information relative to the methods employed and the general ecology of the region may be obtained from this first report. Appreciation is expressed to Dr. Nathan Riser, Woods Hall, Massachusetts, and Dr. Marietta Voge of the University of California at Los Angeles for their assistance in confirming determination of some of the parasitic material.

RESULTS

In total, 538 mammals belonging to 22 species were examined. Of this number, 13 species produced either adult or larval cestodes, or both. In several instances, notably the carnivores, the number of specimens examined was too small to provide representative samples. The fact that no cestodes were found in many of these cases does not mean that none are present in the species. In other cases, many individuals of species such as the chisel-toothed kangaroo rat, *Dipodomys microps bonnevillei* Goldman, were examined without finding a single cestode.

Considering all specimens processed, the prevalence of infection was 8% with adult tapeworms and 6% with larval forms. The prevalence varied considerably among the mammal species. The carnivores, except the spotted skunk, were all infected. The black-tailed jack rabbit was the next highest with 12 out of 19 animals infected. The prevalence also varied in different localities; areas of high population density usually produced larger numbers of parasitized specimens.

The largest number of cestode species was recovered from the black-tailed jack rabbit which was host to 2 adult and 3 larval forms. The coyote was host to 4 species in the study area, all 4 of which were obtained from 2 specimens. Additional coyotes probably would have revealed others. The bobcat had 3 and the badger 1 species. Two rodents, the antelope ground squirrel, *Citellus leucurus leucurus* Merriam, and the desert wood rat, *Neotoma lepida lepida* Thomas both produced 3 species, 1 adult and 2 larval each. *Peromyscus maniculatus sonoriensis*, the most abundant and widespread of all rodents of the region, was host to 2 species, 1 larval and 1 adult. The remainder of the mammals found infected were inhabited by a single species.

The following list presents the cestode species obtained. Pertinent data include observations on hosts, host range, and interrelationships with other species of the desert region. Taxonomic arrangement follows Wardle and McLeod, 1952.

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Order Cyclophyllidea

Family Mesocestoididae

Mesocestoides carnivoricolus Grundmann 1956

Definitive Hosts: Badger, *Taxidea taxus taxus* (Schreber); coyote, *Canis latrans lestes* Merriam; and bobcat, *Lynx rufus pallescens* Merriam. *Range of hosts:* Over the entire region with the exception of the badger which is usually found in areas of heavy rodent population. *Intermediate hosts of larval tetrathyridia:* Deer mouse, *Peromyscus maniculatus sonoriensis* (LeConte), 10 of 115 animals; canyon mouse, *P. crinitus pergracilis* Goldman, 2 of 19 animals. Of these rodent species, the deer mouse is found in all habitats except the barren salt flats. The canyon mouse prefers cliffs and rocky places on the mountain slopes.

Evidence accumulated during this study and from study of material from coyotes from western Utah, indicates that this tapeworm species is distributed throughout the entire Bonneville Basin and has been taken in coyotes from 6 counties of western Utah. In the Dugway area, several sites on the vegetated dunes were located in which deer mice containing tetrathyridia could be taken with regularity. Some of these mice contained as many as several hundred cysts in the liver, lungs and other viscera.

Family Anoplocephalidae

Cittotaenia pectinata americana Douthitt 1915

Definitive Host: Black-tailed jackrabbit, *Lepus californicus deserticola* Mearns. The rabbit is found to range over most of the habitats other than the pickleweed, and perhaps the higher mountains. The intermediate hosts are mites. One rabbit of 19 examined contained 5 tapeworms of this species.

Monoecocestus americana Stiles 1895 and *M. variabilis* Douthitt 1915

Definitive Host: Porcupine, *Erethizon dorsatum epixanthum* Brandt. Both of these species were obtained from a single porcupine. The host is found inhabiting the mountain ranges and may be found frequently on the flatlands. Larval cysticercoids inhabit mites.

Thysanosoma actinoides Diesing 1835

Definitive Host: Mule deer, *Odocoileus hemionus hemionus* Rafinesque. This species is occasionally taken from mule deer that inhabit the mountains of western Utah.

Family Catenotaeniidae

Catenotaenia linsdalei McIntosh 1941

Definitive Hosts: The long-tailed pocket mouse, *Perognathus formosus incolatus* Hall, and the deer mouse, *Peromyscus maniculatus sonoriensis* (LeConte). Adult tapeworms were taken from 7 of 35 pocket mice and 7 of 115 deer mice. *Intermediate Host:* The deutonymph stage of mites.

Hosts containing this species were taken only from small localized areas which are widely separated. In no instance were more than 2 worms present in an individual. At no time were more than one or two infected individuals taken from any locality.

Family Taeniidae

Hydatigera macrocystis Diesing 1850

Definitive Host: The bobcat, *Lynx rufus pallescens* Merriam. *Intermediate Host:* The black-tailed jack rabbit. The bobcat and the rabbit range over most of the study area.

Two rabbits were found to have 5 strobilocerci in the mesentery immediately adjacent to the ileum.

Hydatigera laticollis Rudolphi 1819

Intermediate Host: The black-tailed jack rabbit. Several strobilocerci were recovered from the mesentery of a single rabbit. The adult was not found in the small number of bobcats examined.

Multiceps serialis (Gervais 1874)

Definitive Host: Coyote. *Intermediate Host:* Black-tailed jack rabbit. One coyote examined contained several hundred scolices of this species and 1 jack rabbit, previously reported (Grundmann et al, 1955) contained an enormous cyst containing several thousand scolices. The area from which the rabbit was collected is one that is heavily grazed by sheep.

Taenia rileyi Lowen 1929

Intermediate Hosts: The antelope ground squirrel, *Citellus leucurus leucurus* Merriam, and the desert wood rat, *Neotoma lepida lepida* Thomas. *Incidence:* 2 of 64 ground squirrels and 2 of 31 wood rats. The definitive host of this form is listed as the bobcat although no adult tapeworms were recovered. The larval stages were typical strobilocerci with 33–46 hooks in 2 rows and with an equal division between the number of small and large hooks in all but 1 case. Large hook lengths varied from 176 to 224 microns and the size apparently corresponds to the degree of maturity of the scolex of the larvae with the most mature having the largest hooks. It appears that the growth of the hooks continues until the adult stage is complete.

There is some controversy concerning the validity of the name *Hydatigera lyncis.* Riser (1956) states that it is a composite species and described from scolices of *Taenia* syn. (*Hydatigera?*) *omissa* and the strobila of *T. rileyi.* Furthermore, Riser feels that *T. rileyi* is also a composite having been described from a scolex of *H. laticollis* and a strobila of a new form.

Taenia pisiformis Bloch 1780

Definitive Host: Coyote. *Intermediate Host:* Black-tailed jack rabbit. This species is the most common tapeworm of the coyote. A previous study (Butler and Grundmann, 1954) showed 54.6% of 75 coyotes to be infected. The cysticerci were recovered from 4 of 19 rabbits.

Taenia hydatigena Pallas 1766

Definitive Host: Coyote (not collected during this study but has been previously taken from coyotes collected in Tooele County (Butler and Grundmann, 1954). *Intermediate Host:* Black-tailed jack rabbit and antelope ground squirrel. Cysticerci were obtained from 1 animal each of the intermediate host listed.

Family Davaineidae

Raillietina retractilis Stiles 1925

Definitive Hosts: Black-tailed jack rabbit and Ord kangaroo rat. Adult tapeworms were recovered from 5 of 19 jack rabbits and 3 of 99 kangaroo rats. *Intermediate Host:* Mites.

This species constitutes the most common tapeworm of the jack rabbit in the study area and the rabbit is considered the normal host. Collections of jack rabbits from other desert localities of western Utah also demonsrate a high incidence of infection with this form. The Ord kangaroo rat is probably an incidental host.

Raillietina sp.

Definitive Host: Desert wood rat. *Intermediate Host:* Mites. A single tapeworm was recovered from each of 4 rats of a total of 31 examined.

Family Hymenolepididae Railliet and Henry 1909

Hymenolepis citelli McLeod 1933

Definitive Host: The antelope ground squirrel. Tapeworms were recovered from 7 of 64 animals. In 2 of the cases, so many tapeworms were present that the intestine appeared to be about 3 times its normal diameter and was packed solid with worms. A count in 1 case showed 104 worms in various stages of development.

Hymenolepis sp.

Definitive Host: The grasshopper mouse, *Onychomys leucogaster utahensis* Goldman. A single worm was collected from each of 2 animals of the 6 grasshopper mice examined.

Family Dilepididae Railliet and Henry 1909

Paruterina candelabraria Goeze 1782

Definitive Host: Probably western horned owl, *Bubo virginianus occidentalis* Stone (not collected). *Intermediate Host:* The desert wood rat.

The cysticerci of this species were found in the livers of 6 of 31 wood rats. In all of the cases, the cysticerci were associated with numerous ascarid larvae.

DISCUSSION

The importance and positions of the various rodent species in the food chains of the carnivores were partially elucidated. On the basis of its numbers and wide

ecological distribution, the black-tailed jack rabbit is considered to be the most important intermediate host and prey species of the larger carnivores. The jack rabbit yielded 5 species of larval tapeworms, of which 3 reach their adult stage in coyotes and kit foxes, and 2 reach adulthood in bobcats or cougars.

Of the other rodent species serving as prey for the dominant carnivores, the antelope ground squirrel and the wood rat both served as host for 3 species of cestodes, 1 adult and 2 larval forms in each case. Both were hosts to the strobilocercus of *Taenia rileyi*, a tapeworm reaching its adult stage in the coyote, bobcat and the mountain lion. In addition, larvae of *Taenia hydatigena* were recovered from the ground squirrel and *Paruterina candelabraria* larvae were recovered from the liver of the wood rat.

The deer mouse, the most widespread ecologically and the most abundant of the rodent species in the study area, was also host to 3 species. The deer mouse fits into the food chains of all carnivore species present and along with *P. crinitus*, the canyon mouse, serves as a host for the tetrathyridia of *Mesocestoides carnivoricolus*, the adults of which were recovered from the coyote, bobcat, and badger. A second larval form that has been previously reported from the deer mouse (Riser, 1956) is that of *Taenia rileyi*.

In many localities, especially the vegetated dunes, where a dense, heterogeneous rodent population frequently exists, the prevalence of infection for a species and the parasite population per host increased as the density of the rodent population increased. These areas are excellent for the study of host specificity of the parasitic species present. Since members of most species make contact with parasitic species present through feeding or other habits, the absence of the parasite in a host species living under these conditions likely indicates resistance to infection.

Territoriality and home range of individual rodents, which causes them to feed and move continuously in a prescribed area seems to be a particularly important factor in cases of massive infections. On several occasions, specimens of the antelope ground squirrel were collected in which the intestines were abnormally enlarged and packed almost solid with *Hymenolepis citelli* McLeod. Once the infection becomes established in a locality, it is likely to remain for a number of generations until the area is abandoned because of a rodent die-off or the population reduced to a low point in the population cycle. Home range studies on small mammals have been treated extensively in papers by Blair (1940), Burt (1943) and Howell (1954), et al.

Studies on the relation of parasitism to migratory habits of hosts, to isolation of populations, and to food habits have been described in the report on the nematode parasites of this region Grundmann (1957).

SUMMARY

A study was made of the cestodes inhabiting 22 species of common mammals collected from the Great Salt Lake Desert region of western Utah. Of these, 13 mammal species of the group produced 17 species of cestodes. The prevalence of infection of 538 specimens dissected was approximately 8% with adult forms, and 6% with larval stages. The few specimens of the common carnivores of the region, namely, the coyote, bobcat and badger, that were examined revealed a high prevalence of parasitism. In the mammals other than carnivores, such as the rabbits and rodents, the largest number of tapeworm species was taken from the wide-ranging

black-tailed jackrabbit, *Lepus californicus deserticola* Mearns, which proved host to 5 species. Three cestode species each were collected from the antelope ground squirrel, *Citellus leucurus leucurus* Merriam and the desert wood rat, *Neotoma lepida lepida* Thomas. The deer mouse, *Peromyscus maniculatus sonoriensis* (LeConte), the most widely distributed and abundant of the rodents in the area, was host to 2 species. The remaining infected mammal species possessed only a single cestode species each.

Of the 17 species of cestodes, 3 were present only as larval stages in intermediate hosts. The dissection of an additional number of carnivores probably would have revealed the adults. Six species were recovered as adults; the larvae of these are generally assumed to be cysticercoids in the deutonymph stages of mites. Both larval and adult forms were recovered for the remaining 8 species.

Distribution of infection was found to be fairly uniform among widely ranging species. Host species with greater restriction to habitat demonstrated an intermittent type of distribution with infection in some localities but none in others. Population density, home range and territoriality were considered as important factors affecting prevalence in a locality and rate of distribution.

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AMBLYOMMA IMITATOR, A NEW SPECIES OF TICK FROM TEXAS AND MEXICO, AND REMARKS ON THE SYNONYMY OF *A. CAJENNENSE* (FABRICIUS) (ACARINA–IXODIDAE)

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Re-examination of extensive tick collections in the Rocky Mountain Laboratory labeled *"Amblyomma cajennense,"* and study of numerous collections received for identification, have revealed a new species, strongly resembling *A. cajennense,* in southern Texas and Mexico. The new form is described below. Measurements are in millimeters.

Amblyomma imitator n. sp.

Holotype: Female, from goat, Brownsville, Texas, February 8, 1934 (RML 10021) L. Demieville. In the Rocky Mountain Laboratory.

Allotype: Male, data as for holotype. In the Rocky Mountain Laboratory.

Paratypes: 3 males, 5 females, 7 nymphs with same data as holotype. 14 males, 12 females, from cow, Brownsville, Texas, May 18, 1935 (RML 11087) L. Demieville. 3 males, 7 females, 1 nymph, from man, El Bañito, near Valles, San Luis Potosí, México, 1939 (RML 17459) R. Traub. Paratypes are deposited in the U. S. National Museum, Chicago Natural History Museum, Laboratorio de Entomología de Instituto de Salubridad y Enfermedades Tropicales, México, D.F., and the Rocky Mountain Laboratory.

Over 700 other specimens examined, designated by the accession numbers assigned to them at the Rocky Mountain Laboratory, are as follows: 10036, 10048, 11049, 11060, 11063, 32241, 34576, 34585, 32165, 32172, 32175, 32180, 32178, 32169, 25328, 25329, 15492, 15493, 15494, 17470, 17467, 17458, 17461, 17465, 17468, 29974, 24010, 24011, 24015, 32160, 32176. These are all from Texas and Mexico, and from these hosts: man, dogs, horses, burros, cattle, goats, deer, peccary, opossum, *Sciurus aureogaster, Meleagris gallopavo, Geococcyx californianus;* some were from vegetation or the ground.

Female: Resembles *A. cajennense* but tends to be smaller; differs particularly from it in lacking chitinous tubercles at the postero-internal angle of the festoons, and in details of the external genitalia. Length of holotype, excluding capitulum, 3.18; width 2.56. Length and width of largest and smallest unfed specimens seen, 3.49 by 2.67 and 2.87 by 2.15, respectively. Contour oval, broadest at about middle. Color of unfed specimens paler than usual in *Amblyomma.* Largest fed specimen seen is 9.74 long by 6.66 wide and both ends are equally broad.

Capitulum: Length (holotype) 0.98; width of basis, 0.63. Details as in *A. cajennense.*

Scutum: Triangular, 1.57 long by 1.70 wide (holotype); size ranges from about 1.34 by 1.52 to 1.84 by 1.90. Shape, grooves and punctations as in *A. cajennense.* Ornamentation variable in distinctness and extent; pattern, when well developed, as in Figure 1B, similar to that of *A. cajennense,* Figure 1D.

Legs and Spiracular Plate: As in *A. cajennense.*

Genital aperture: Located between coxae II as in *A. cajennense.* Genital apron overlaid posterolaterally on either side by a blunt flattened projection darker in color than the apron and adjacent integument, as illustrated in Figure 2A. In *A. cajennense* these projections are long and narrow as shown in Figure 2B. This differentiating character is easily observed in clean, unfed or fed, unmounted specimens properly lighted under 36× magnification.

Male: Closely resembles *A. cajennense* and not always distinguishable from it with certainty but usually paler and tending to be narrower and smaller. Ornamentation variable in extent and definition; pattern, when well developed, as in Figure 1A and similar to that of *A. cajennense* as shown in Figure 1C. Length of allotype, excluding capitulum, 2.97; width 2.36. Largest and smallest specimens seen are 3.48 by 2.66 and 2.26 by 1.77, respectively.

Nymph: Similar to *A. cajennense* except scutum shagreened instead of smooth and punctations somewhat larger, deeper, and more distinct.

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Although *A. imitator* has its closest affinities with *A. cajennense* with which it has been compared, it also resembles *A. oblongoguttatum* Koch, 1844 and *A. tapirellum* Dunn, 1933. The female of *A. imitator* is readily distinguished from that of *A. oblongoguttatum* by the more widely spaced spurs of coxa I, the external being of medium length and the internal no more than half as long, and the genital

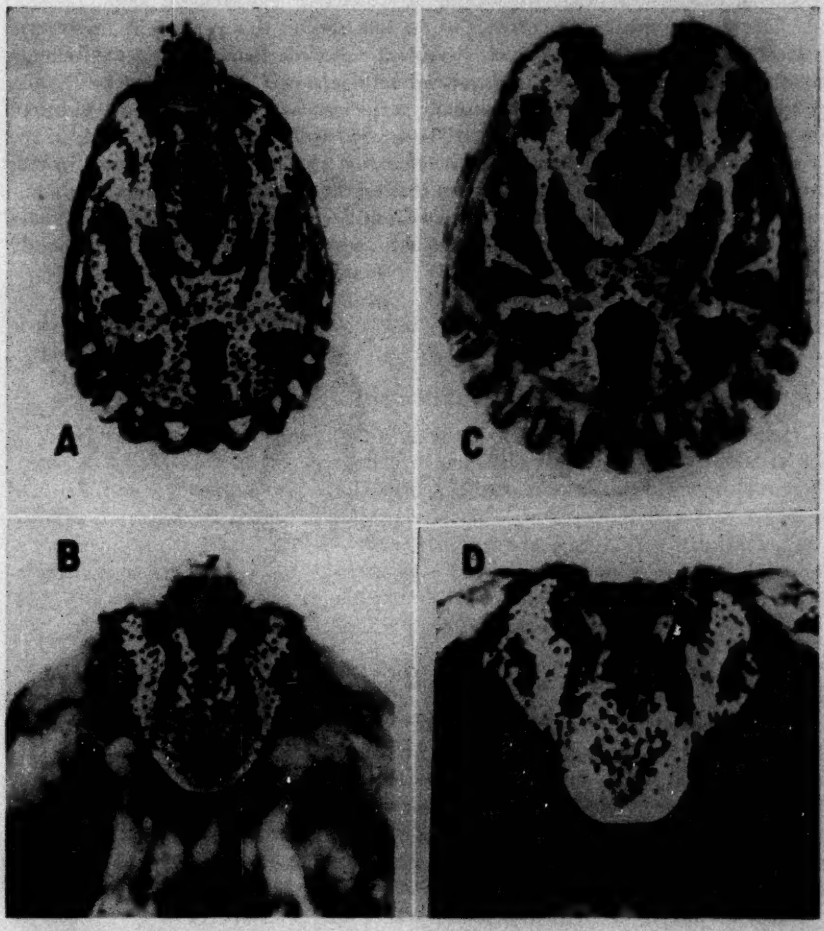

FIGURE 1. Well-ornamented scutums of *Amblyomma imitator*. (A—male, B—female) and *A. cajennense* (C—male, D—female).

area. Females of *A. tapirellum* have the genital area much as in *A. imitator* but the spurs of coxa I are subequal and longer. The male of *A. imitator* has widely spaced and markedly unequal spurs on coxa I, as in the female, and is thus easily distinguished from *A. oblongoguttatum* and *A. tapirellum* in which these spurs are closer together and subequal. *A. imitator* and *A. oblongoguttatum* are comparable in size and much smaller than *A. tapirellum*.

The distributional range of *A. imitator*, from southern Texas to Vera Cruz, Mexico, is within that of *A. cajennense* but the latter continues southward as far as Buenos Aires, Argentina. *A. oblongoguttatum* occurs from Oaxaca, Mexico, to Brazil according to Robinson (1926) and unpublished data of the Rocky Mountain Laboratory. Material at hand of *A. tapirellum*, until now reported only from Panama, extends its range to Nicaragua, Colombia, and Venezuela.

The possible medical importance of the new species is suggested by the several collections from man. Moreover, it is now apparent that the Rocky Mountain spotted fever transmission tests performed with supposed *A. cajennense* by Parker, Philip, and Jellison (1933) were actually performed with *A. imitator*, as evidenced by sample specimens still on hand. The several collections from livestock in the vicinity of Brownsville, Texas, indicate that it is of veterinary importance in that area. We have no *A. cajennense* from that locality and conversely no *A. imitator* from the Kingsville area where *A. cajennense* is common. Since the new species has been confused with *A. cajennense* until now, further collecting is obviously needed to determine its distribution.

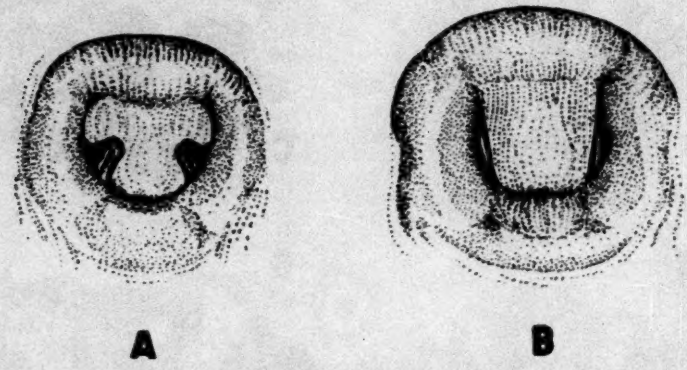

FIGURE 2. Female genital area of *Amblyomma imitator* (A) and *A. cajennense* (B).

The similarity of the new species to *A. cajennense* necessitated ascertaining whether the new species may have been previously described under one of the several synonymic names of *A. cajennense* listed by Robinson (1926) and Cooley and Kohls (1944). Koch's types of *A. mixtum*, *A. tenellum*, and also of *A. cajennense* (Fabricius' type is lost), were made available through the courtesy of Prof. A. Kaestner of the Zoological Museum, Berlin. After examining these specimens, Tonelli-Rondelli (1937) concluded that *A. mixtum* and *A. tenellum* were valid species distinct from *A. cajennense*. Aragão and Fonseca (1953) returned *A. mixtum* and *A. tenellum* to synonymy, apparently without examining the types, maintaining that Tonelli-Rondelli failed to consider the variability observed in *A. cajennense* when extensive materials from laboratory rearings and from various sources in nature are studied.

Koch's types of *A. cajennense*, 1♂, 1♀, labeled "8491 cajennense type Brasilien, Cayenne" and 1♀ labeled "8492 cajennense type Mexico Deppe. Koch," agree in all particulars with *A. cajennense* as currently interpreted. Those of *A. mixtum*, 3♂, 1♀, labeled "1061 mixtum type Mexico Deppe.", are clearly *A. cajennense* and

I concur with Aragão and Fonseca in returning the names to synonymy. The types of *A. tenellum*, 2♂ labeled "1043 tenellum type Mexico Deppe.", are pale and small (scutums 2.27 by 1.77 and 2.15 by 1.64, respectively) and are suggestive of *A. imitator* but I am inclined to agree with Aragão and Fonseca that they are *A. cajennense*. The name *A. tenellum* may apply to the new species but to date characters for positive identification of all males of the latter have not been discovered.

Though synonymized under *A. cajennense* by authors, *Ixodes crenatus* Say, 1821 (Southern States, U.S.A.) is unrecognizable from the description and the types are no longer in existence. Whether *I. herrerae* Dugès, 1891 (Mexico) is *A. cajennense*, as regarded by previous authors, or another species cannot be determined from the description, and no specimens are available for study.

Amblyomma sculptum Berlese, 1888 (Brazil), *A. parviscutatum* Neumann, 1899 (Brazil), and *A. versicolor* Nuttall and Warburton, 1908 (Mexico) are undoubtedly *A. cajennense*, as previous authors had determined. However, Tonelli-Rondelli (1937) sought to re-establish *A. sculptum*, and in addition described *A. finitimum* and *A. tapiri* from tapirs in British Guiana as new species, but again I agree with Aragão and Fonseca (1953) in reducing all three of these as synonyms of *A. cajennense*.

SUMMARY

Amblyomma imitator, a new species strongly resembling *A. cajennense* (Fabr.), recorded from man and a wide variety of domestic and wild animal hosts, is described from southern Texas and Mexico. Characters distinguishing it from *A. cajennense* and from *A. oblongoguttatum* Koch and *A. tapirellum* Dunn which it also resembles are given. Review of the synonymy of *A. cajennense*, including examination of Koch's types of *A. cajennense* (Fabricius' material lost), *A. mixtum*, and *A. tenellum*, revealed that, with the possible exception of *A. tenellum*, none of the synonymic names are available for the new species.

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WINTER SURVIVAL OF SOME CATTLE PARASITES ON A KENTUCKY PASTURE WITH OBSERVATIONS ON THE EFFECTS OF LOW-LEVEL PHENOTHIAZINE TREATMENT*

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Information on the survival on pastures of the free-living stages of the various species of gastro-intestinal parasites has considerable practical application to the problem of control. Numerous investigations, which were reviewed recently by Kates (1950) have been made on sheep nematodes under a variety of conditions both in the laboratory and in the field in different areas of this country. In contrast, relatively little information is available on the related species infecting cattle (Goldberg and Rubin, 1956; Porter et al, 1941; Swanson, 1942, 1946).

In Kentucky, the longevity of the intermediate stages of sheep parasites on pasture has been studied during summer (Doll and Hull, 1948) and winter (Todd, et al, 1949). The present observations on cattle parasites were made as part of a general study on the occurrence and control of gastro-intestinal parasites of dairy animals in this area.

MATERIALS AND METHODS

Observations were made over 4 consecutive winters, 1953–1957, on pasture that was predominantly Kentucky blue-grass. Prior to these investigations the pasture was utilized for a number of years as a summer grazing area for the heifer calf replacements for the station dairy herd. During the study the heifers were removed at the close of each summer grazing season, no additional manure was applied to the pasture, and no animals had contact with the pasture during the winter intervals. These intervals were: 7 October 1953 to 10 May 1954; 20 September 1954 to 20 April 1955; 28 September 1955 to 2 May 1956; 17 October 1956 to 1 May 1957. After the first winter, which followed the 1953 grazing season during which no anthelmintic treatment was used, the pasture was divided into 2 similar parts and each heifer on one part (Pasture A) was fed 1.5 gm phenothiazine in the daily grain ration during the 1954 grazing season. A similar group of heifers on the other part (Pasture B) was maintained as untreated controls. This experiment was repeated during the 1955 and 1956 grazing seasons by a similar grouping and treatment of the respective crops of heifers except that the daily dose of phenothiazine was increased to 2.0 gm per animal on Pasture A for the 1956 season.

The test animals (sample calves) for these winter survival trials were 3- and 4-month-old male Holstein-Friesians that were raised helminth-free by confinement from birth in elevated cages. One of the sample calves was placed on each pasture for a 7-day period at the close of each winter interval, which was also the start of the ensuing grazing season. After the 1-week period on pasture, the calves were removed to elevated, wire-bottom cages for a 4-week holding period, after which they were killed and the gastro-intestinal tracts examined to determine the number and species of parasites present.

Determinations of the small worms (*Ostertagia, Nematodirus, Cooperia, Trichuris,* and *Trichostrongylus*) were made by a careful inspection at 3× magnification of replicated, small samples (50 or 100 ml) comprising a minimum of 10% of the total contents and washings of the abomasum, small intestine, and large intestine. Preliminary digestion of the abomasal wall in artificial gastric juice was included in the search for *T. axei* and imbedded *O. ostertagia.* After sampling, the remainder of the gastro-intestinal contents was washed (40-mesh screen) and examined for the larger forms (*Haemonchus, Bunostomum, Moniezia,* and *Oesophagostomum*).

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RESULTS AND DISCUSSION

The number and species of worms recovered from the sample calves are presented in Table I, and the climatological data on temperature and precipitation for the 4 winter intervals are given in Table II.

TABLE I. *Worm parasites recovered from sample calves on pastures A and B for the indicated periods following the 1953–54, 1954–55, 1955–56, and 1956–57 winter intervals.*

Species	10–17 May 1954		20–27 April 1955		2–9 May 1956		1–7 May 1957	
	No.*	%	No.	%	No.	%	No.	%
Pasture A								
Ostertagia ostertagi	60	2	10	1	120	2	0	0
Nematodirus helvetianus	2015	71	560	65	5,820	77	6,690	92
Cooperia oncophora	755	27	130	15	1,170	15	50	1
Cooperia punctata	0	0	0	0	70	1	0	0
Trichuris sp.	0	0	165	19	400	5	490	7
Moniezia sp.	0	0	0	0	0	0	0	0
Pasture B								
Ostertagia ostertagi	60	2	135	15	300	8	785	16
Nematodirus helvetianus	2015	71	180	19	1,190	32	2,820	58
Cooperia oncophora	755	27	430	46	2,030	54	740	15
Cooperia punctata	0	0	0	0	0	0	0	0
Trichuris sp.	0	0	190	20	240	6	560	14
Moniezia sp.	0	0	0	0	1	—	1	—

* Average for 2 worm-free calves placed on pasture before treatment started.

From Table I it will be noted that *Ostertagia ostertagi, Nematodirus helvetianus,* and *Cooperia oncophora* survived all 4 winters. *Trichuris sp.* was recovered following 3 of the winters, and *Cooperia punctata* was present in 1 of the sample calves following the 1955–56 winter. *Moniezia* sp. was recovered after 2 of the winter intervals.

Survival of *O. ostertagi* and *N. helvetianus* is in accord with other reports on the survival of these and related species parasitizing sheep and cattle (Goldberg and Rubin, 1956; Kates, 1950). Both *Ostertagia* and *Nematodirus* have been generally characterized by a high level of resistance to cold weather.

The ability of *Cooperia oncophora* to survive the winter months on pasture, which was also recorded by Goldberg and Rubin (1956) is of special interest because it appears to be at variance with several observations on *C. curticei* (Kates, 1950), although there is 1 record (Baker, 1939) of *C. curticei* surviving for a year on pasture in New York. Survival of another species, *C. punctata,* was observed over 1 winter, although other unpublished observations established the presence of relatively small numbers of this species on both pastures A and B during the 1954, 1955, and 1956 grazing seasons. Thus, considerable variation

TABLE II. *Climatological data* for winter survival studies.*

Month	1953–54		1954–55		1955–56		1956–57		50-year Average	
	Temperature†	Precipitation‡	Temperature	Precipitation	Temperature	Precipitation	Temperature	Precipitation	Temperature	Precipitation
September	69.9	1.58	73.6	4.97	72.6	2.43	65.9	1.77	69.2	2.77
October	61.8	1.12	58.0	1.33	56.9	2.29	61.0	1.78	57.6	2.39
November	46.3	1.46	44.6	2.36	41.7	2.56	43.2	2.32	44.6	3.15
December	35.0	2.44	35.2	3.38	33.6	1.86	44.4	4.52	36.1	3.62
January	35.3	4.16	32.1	1.57	31.5	3.25	31.4	4.45	34.0	4.44
February	42.9	1.68	36.4	7.69	40.6	7.06	40.3	3.48	35.4	3.25
March	42.8	2.38	46.5	7.03	43.6	6.51	43.5	2.06	44.1	4.58
April	60.8	3.79	59.0	1.71	51.7	4.79	57.9	7.66	54.0	3.53

* Recorded by the U. S. Department of Commerce, Weather Bureau, Lexington, Kentucky.
† Average monthly Temperature (° F).
‡ Inches.

of ability to survive environmental factors appears to exist among members of the genus *Cooperia*.

In this testing procedure the failure to recover a species from the sample calves is not prima facie evidence of lack of survival ability, but the negative findings are of value as an index of practical significance in determining control programs. In the present observations the over-winter survival was in agreement with the order of dominance of these parasites in the heifers during the grazing seasons. In addition to the 5 species of nematodes in Table I that survived, 2 species (*Haemonchus* sp. and *Oesophagostomum radiatum*) were present on the pastures during the 1954 grazing season and *Haemonchus* sp. was present during the 1955 season. Both of these species were present in small numbers, and others (Kates, 1950) indicate these genera have a low order of resistance to environmental factors.

Only immature specimens of whipworms (*Trichuris* sp.) were recovered from the calves and these were identified only to genus (Table I). Viability of these forms over winter is in agreement with the extreme resistance generally accorded to members of this genus (Goldberg and Rubin, 1956; and Kates, 1950).

Although only immature specimens of *Moniezia* sp. were recovered from 2 calves in the present trials, the over-winter survival of these forms has also been recorded by others (Stoll, 1935). Tapeworm eggs were present in the heifers grazing both A and B pastures during the 1953, 1954, 1955, and 1956 seasons, and only *M. expansa* was known to be present.

The climatological data in Table II show that the most significant departure from normal was the deficiency in precipitation during the 1953–54 interval. Both 1953 and 1954 were drouthy years, but the unusually heavy rainfall for February and March 1955 offset the relatively dry portions of the 1954–55 winter and produced an average for the interval in excess of the 50-year average.

In this experimental set-up, the number and species of worms in the sample calves from Pasture A would indicate survival and the efficacy of medication. Furthermore, effects of treating successive groups of heifers on Pasture A during the 1954, 1955, and 1956 summer grazing seasons would tend to be cumulative. None of the species in this study have been regarded as particularly susceptible to the action of low-level phenothiazine medication (Andrews et al, 1943, Brock et al, 1952; Cauthen, 1953; Dennis et al, 1955; Herlich and Porter, 1954; Shelton et al, 1956; Turk et al, 1954). Thus, the alteration of the worm population brought about by the 3 years of medication of the heifers on Pasture A was an unexpected development. The changes were not fully evident in these survival trials until the last over-winter interval (1956–57) as indicated by the worms found in the calves on the pasture in May 1957 (Table 1). Treatment of the grazing animals during the preceding summer (1956) was at the 2.0-gm daily dose level and resulted in the complete elimination of *O. ostertagi* and a marked reduction in the numbers of *C. oncophora* on Pasture A. In contrast, the number of *N. helvetianus* increased in the presence of this treatment. Treatment of the animals on Pasture A during the 1954 and 1955 grazing seasons at the 1.5-gm level was not nearly so effective as reflected by the worms found in the calves on pasture in April 1955 and May 1956, but the trends toward a decrease of *O. ostertagi* and *C. oncophora*, and an increase of *N. helvetianus* were evident.

No activity of drug against *Trichuris* sp. was evident, while the numbers of *C. punctata* and *Moniezia* were too small for an evaluation.

The species relationships in these calves are in accord with unpublished observations made on additional worm-free calves placed on the pastures at intervals during the several grazing seasons.

Thus, the species-selective action by this type of medication is of interest not only because of the effective control of 2 species, *O. ostertagi* and *C. oncophora*, but also because of the lack of action in controlling *N. helvetianus*. Indeed, control of the former 2 species appeared to favor the development and survival of *N. helvetianus*. This appears to be a hitherto unobserved result of low-level phenothiazine treatment. The predominance taken by *N. helvetianus* may be accounted for by the "room to grow" concept (Taylor, 1957) resulting from the elimination of competitive species or by direct "stimulating" action by the drug on *N. helvetianus*.

SUMMARY

Observations over 4 winter intervals (1953–54 through 1956–57) averaging 7 months in length indicated the survival of the free-living stages of *Ostertagia ostertagi, Nematodirus helvetianus, Cooperia oncophora, C. punctata, Trichuris* sp., and *Moniezia* sp. on blue-grass pasture in central Kentucky. There was no evidence that *Haemonchus* sp. and *Oesophagostomum radiatum* over-wintered on pasture. Low-level phenothiazine medication of successive groups of heifers on 1 pasture during the intervening summer grazing seasons showed a species-selective action on the nematodes. Of the 3 predominant species, *O. ostertagi* and *C. oncophora* were effectively controlled, but the development and survival of *N. helvetianus* were favored. These effects of treatment were reflected in the numbers of each of these species that survived the succeeding winter intervals on the treated pasture in comparison with the control.

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RESEARCH NOTE

FAILURE OF THE EXCRETORY DUCTS OF A TAPEWORM (*HYMENOLEPIS DIMINUTA*) TO TRANSPORT ABSORBED SUBSTANCES FROM ONE PORTION OF THE WORM TO ANOTHER.

The observation of von Brand (1933, Ztschr. Vergleich. Physiol. **18**: 562–596) of fat droplets in the lumen, and that of R. M. Hedrick (1958, J. Parasitol.: 75–84) of heavy deposits of phospholipid around the walls of the excretory ducts of tapeworms, suggested the possibility of transport by these ducts of absorbed materials from one part of the worm to another, a matter which might be of some importance in view of the gradients of metabolism and of uptake of metabolites by tapeworms, reported by various workers.

This transportation was tested on adult *Hymenolepis diminuta* obtained from laboratory-infected white rats. As soon as possible after the worms had been flushed from the intestine, washed, and blotted, they were laid on chilled agar in an ice-cube tray with about 1/10 of the anterior end immersed in a dish containing a few ml of an isotonic solution to which had been added a radioactive substance (P^{32} phosphate, S^{35}-labelled methionine, or Ca^{45} chloride) and the posterior end in a small vial containing 1 ml of isotonic solution. The dishes, notched to allow passage of the worms over the lips, had been set in the agar before this had hardened, and the agar built up around the notches. The parts of the worms between the dish and the vial were then covered by melted and cooled agar so that they were quickly imprisoned, although in spite of this they succeeded in squirming forward until, in some cases, 1/4 to 1/3 of the anterior end became free in the dish. As soon as the agar had hardened, the whole tray was placed in a water bath at 38°C.

After periods of from 45 minutes to 4 or 5 hours the worms were cut, in situ, into 4 sections—(1) the end of the worm immersed in the radioactive solution, (2) the adjacent half of the imbedded portion, (3) the distal half of the imbedded portion, and (4) the portion in the vial of isotonic solution. The 4 portions of the worms were washed in 6 changes of isotonic solution and, along with the 1 ml of isotonic fluid in the vial, appropriately treated for determination of radioactivity. Counting was done in a gas flow counter long enough to give a fluctuation error of less than 2%. Corrections were made for background, but not for self-absorption. The agar from which the worms had been removed was checked for radioactivity by means of a Geiger counter with negative results.

Sixteen worms were tested: 5 with their anterior ends in P^{32} phosphate solution, 4 in S^{35}-labelled methionine, and 3 in Ca^{45} chloride; and 4 with their posterior ends in the S^{35}-labelled methionine solution. All showed uptake in the immersed portions, although the actual counts were very erratic, probably due in part to variations in self-absorption. There was, however, no evidence of any transportation of either P^{32} or Ca^{45}; out of 32 readings there were only 3 very low positive counts, sporadic in distribution, and obviously due to contamination. In the methionine tests most of the portions of the worms adjacent to the immersed portions showed some activity, but the counts were only a small fraction of the counts of the immersed portions. It seems evident that this activity resulted from some slight diffusion, rather than transportation via the excretory ducts. In only 1 of the 16 tests did the fluid in the distal tube give a small positive count, and this was obviously a contamination since in this case all of the 3 non-immersed parts of the worm were negative.

These results fail to give evidence for transportation of any appreciable amount of absorbed substances from one part of *H. diminuta* to others via the excretory ducts. This work was supported in part by research grant E1384 from the National Institutes of Health, Public Health Service.—MARY MACPHERSON, *Rice Institute, Houston, Texas.*

CYRTOSOMUM READI N. SP. AND *CYRTOSOMUM HEYNEMANI* N. SP. (OXYUROIDEA; ATRACTIDAE) TWO NEW PINWORMS OF IGUANIDS*

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Recently, the author had occasion to examine the cecal contents of several hundred North American Iguanidae for their pinworm infections (Gambino, 1957b). The major portion of the specimens examined are part of a large series of North American Iguanidae in the Herpetological Collection at the University of California at Los Angeles.[1] In addition, a considerable number of specimens collected by the author and others[2] from the coastal mountains and deserts of southern California were examined.

TABLE I. *Summary of iguanid hosts examined for infection with* Cyrtosomum *spp., incidence of infection, and species of parasite found.*

Host	Number Examined	Number Infected	Parasite
Anolis carolinensis Voigt	60	0	—
Basiliscus vittatus Wiegmann	14	2	*C. scelopori*
Callisaurus draconoides Blainville	80	27	*C. penneri*
Crotaphytus collaris (Say)	22	8	*C. heynemani* & *C. readi*
Ctenosaura hemilopha (Cope)	2	1	*C. scelopori*
Ctenosaura similis (Gray)	2	1	*C. scelopori*
Dipsosaurus dorsalis (Baird and Girard)	30	7	*C. scelopori*
Enyaliosaurus quinquecarinatus (Gray)	5	3	*C. scelopori*
Gambelia wislizenii Baird and Girard	71	14	*C. readi*
Holbrookia maculata Girard	48	23	*C. heynemani*
Holbrookia texana (Troschel)	39	2	*C. heynemani*
Iguana iguana (Linnaeus)	10	0	—
Phrynosoma asio Cope	2	1	*C. readi*
Phrynosoma m'calli Hallowell	8	1	*C. readi*
Phrynosoma modestum Girard	6	0	—
Phrynosoma platyrhinos Girard	6	3	*C. readi*
Phrynosoma solare Gray	5	3	*C. readi*
Sauromalus obesus (Baird)	12	4	*C. scelopori*
Sceloporus clarki Baird and Girard	13	6	*C. heynemani*
Sceloporus graciosus Baird and Girard	20	2	*C. penneri*
Sceloporus magister Hallowell	10	1	*C. penneri*
Sceloporus occidentalis Baird and Girard	72	16	*C. penneri*
Sceloporus orcutti Stejneger	8	0	—
Sceloporus poinsettii Baird and Girard	30	1	*C. heynemani*
Sceloporus scalaris Wiegmann	1	0	—
Sceloporus torquatus Wiegmann	5	0	—
Sceloporus undulatus (Daudin)	63	2	*C. heynemani* & *C. readi*
Sceloporus woodi Stejneger	7	1	*C. heynemani*
Streptosaurus mearnsi (Stejneger)	2	0	—
Uma scoparia Cope	10	9	*C. penneri*
Urosaurus auricularis (Cope)	8	0	—
Urosaurus bicarinatus (Dumeril)	4	0	—
Urosaurus microscutata (Van Denburgh)	6	0	—
Urosaurus ornatus (Baird and Girard)	7	0	—
Uta graciosa (Hallowell)	4	0	—
Uta stansburiana (Baird and Girard)	57	1	*C. penneri*

Several oxyuroid genera were represented among the pinworms found during these examinations. However, specimens which could be referred to the atractid genus *Cyrtosomum* Gedoelst, 1919 were responsible for most of the infections. A summary of the iguanid hosts examined and the incidence of infection with *Cyrtosomum* spp. appears in Table I.

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* Data presented in this paper was taken from a thesis submitted in partial satisfaction of the requirements for the degree of Doctor of Philosophy, University of California at Los Angeles.

[1] The author wishes to acknowledge the kindness of Dr. Raymond B. Cowles for making these specimens available.

[2] The aid of Mr. Hisao P. Arai, Mr. John J. Deelman, Miss Velma Vance and Mr. James Warren is gratefully acknowledged.

Detailed study of a large number of these atractids revealed 4 distinct morphological types. One of the 4 morphological forms was identified by the author earlier as *C. scelopori* Gedoelst, 1919 (Gambino, 1957a). A second form was described by the author as a new species, *C. penneri* Gambino, 1957. Two others, considered new species by the author, are described below. An emended generic diagnosis of *Cyrtosomum* must accompany these descriptions.

Genus *Cyrtosomum* Gedoelst, 1919

Emended Diagnosis: Oxyuroidea; Atractidae Travassos, 1919. With characters of subfamily Atractinae Raillet, 1919.

Small worms, body cylindrical and attenuated at extremities. Anterior extremity rounded or truncate. Mouth terminal, small, with 6 lips of approximately equal size; 2 subdorsal, 2 subventral, and 2 lateral. The subdorsal and subventral lips each bear 1 or 2 papillae; if 1, then a single dorsal and a single ventral interlabial papilla occurs. Each lateral lip bears 1 papilla medially and 1 amphid laterally. Buccal capsule extremely small and embedded in anterior portion of esophagus. Cuticle without alae or other modifications except for extremely minute transverse cuticular striations. Tail conical in both sexes.

Esophagus bipartite, with posterior portion shorter than, or equal to, anterior portion. Y-shaped lumen of corpus with accessory canals at margins. Posterior portion of esophagus terminates in a bulb with valvular apparatus. Intestine expanded anteriorly, attenuated posteriorly and ending abruptly a short distance from cloacal or anal opening. A short rectum joins intestine to cloaca or anus. Three rectal glands surround the rectum. Nerve-ring crosses at junction of anterior with posterior esophagus.

Excretory system of "H" type, with 2 anterior and 2 posterior lateral canals leading to a moderate-sized bladder. Excretory pore opens to the exterior just anterior to esophageal bulb.

Vulva located in posterior region of body just anterior to anus. Vagina directed anterodorsally from vulva. Proximal portion of vagina strongly musculatured. Single uterus forms large sac-like structure which, in gravid worm, contains developing embryos or fully developed juvenile worms. Short muscular oviduct connects uterus with ovary. Ovary flexed, with tip directed posteriad.

Vas deferens directed anteriorly and dorsally from cloaca, then joins seminal vesicle which extends anteriorly in dorsal region of body. Seminal vesicle joins testis shortly before latter flexes. Extremity of testis directed posteriad. Two spicules may be equal, sub-equal, or unequal. Both spicules sharply pointed distally. No gubernaculum or accessory piece present.

Number of male caudal papillae variable. Two or 3 pairs of pre-cloacal papillae; 1 ad-cloacal pair, 2 pairs immediately post-cloacal, and 3 pairs on distal portion of tail. Pre-cloacal papillae ventral. First 2 post-cloacal pairs lateral. Remaining post-cloacal pairs on distal portion of tail with 2 pairs ventral and third pair dorso-lateral. Ad-cloacal and first post-cloacal pairs may be lacking.

Host: Iguanidae
Location: Cecum
Locality: North America

Each of the following species descriptions is based on 20 specimens from an individual host specimen. Ten worms of each sex were carefully selected as fully mature representative specimens. These specimens were selected only in the sense that large worms showing gravid reproductive systems were used. The arbitrary number of 10 specimens of each sex was considered sufficient to encompass properly the normal range of variation in a particular species of host, considered the "type host."

For each measurement the range, given in parentheses, is preceded by the mode and followed by the mean. All measurements are in millimeters. Each specimen was cleared in lacto-phenol before measurement.

The iguanid genera *Crotaphytus, Gambelia* and *Phrynosoma* were found to share a pinworm parasite of the genus *Cyrtosomum*. The worms found parasitizing these 3 genera are morphologically distinct from forms parasitizing other genera of Iguanidae. Sufficient observations and comparisons with other *Cyrtosomum* spp. were made to warrant consideration of the differences as of specific magnitude.

The name *Cyrtosomum readi* n. sp. is proposed for this new species in honor of Dr. Clark P. Read of the Johns Hopkins University.

Of 71 specimens of the leopard lizard, *Gambelia wislizenii* (Baird and Girard) examined, 14 harbored this new species. A lesser number of specimens (27) of *Phrynosoma* spp. yielded 8 infections of *C. readi* n. sp. One specimen of 22 *Crotaphytus collaris* Say examined had a single infection of *C. readi* n. sp., while 3 other specimens of *C. collaris* were infected with both *C. readi* n. sp. and *C. heynemani* n. sp. (described below). The 1 infection of *C. readi* n. sp. which was found in *Sceloporus undulatus* Say is considered an incidental infection.

Cyrtosomum readi n. sp.
(Plate I)

Diagnosis: Atractidae Travassos, 1919, with characters of the genus *Cyrtosomum* Gedoelst, 1919.

Anterior extremity rounded. Subdorsal and subventral lips each bear an extensible knobbed papilla. Lateral lips bear a medial papilla and a lateral amphid. A single medio-dorsal and a single medio-ventral papilla occur. Male: 1.68 (1.60–1.84) 1.72 long, 0.096 (0.080–0.112) 0.098 wide at level of esophageal bulb. Esophagus 0.352 (0.352–0.348) 0.359 long. Anterior muscular portion 0.208 (0.208–0.216) 0.209 long, glandular portion 0.144 (0.128–0.168) 0.150 long. Ratio esophagus length to body length less esophagus length .26 (.24–.28) .26. Ratio esophageal parts .69 (.69–.78) .72. Esophageal bulb 0.072 (0.056–0.080) 0.072 long by 0.072 (0.064–0.080) 0.072 wide. Cloaca 0.144 (0.128–0.160) 0.143 from posterior extremity. Spicules extremely unequal in length, sharply pointed distally. Right spicule 0.096 (0.088–0.096) 0.094, left spicule 0.192 (0.192–0.204) 0.194. Spicule ratio .50 (.46–.50) .49. Ratio left spicule length to body length .11 (.10–.13) .11.

Seven pairs of caudal papillae: 3 pairs subventral and pre-cloacal, 1 pair lateral and post-cloacal; on distal portion of tail, 2 pairs ventral, 1 pair latero-dorsal. Female: 1.85 (1.70–2.00) 1.86 long. 0.112 (0.096–0.128) 0.111 wide at level of esophageal bulb. Esophagus 0.352 (0.344–0.368) 0.354 long. Muscular anterior portion of esophagus 0.200 (0.192–0.208) 0.199, glandular portion 0.152 (0.152–0.160) 0.155 long. Ratio esophagus length to body length less esophagus length .23 (.22–.25) .24. Ratio esophageal parts .78 (.77–.81) .78. Esophageal bulb 0.072 (0.064–0.080) 0.074 long, by 0.072 (0.064–0.080) 0.074 wide. Anus 0.192 (0.144–0.240) 0.195 from posterior extremity. Vulva 0.096 (0.080–0.112) 0.094 from anus and 0.288 (0.240–0.336) 0.289 from posterior extremity.

Type Host: Gambelia wislizenii silus (Stejneger) (In Herpetological Collection, University of California at Los Angeles)

Location: Cecum

Type Locality: Arvin, Kern County, California

Type Specimens: Holotype: Male. Allotype: Female. U. S. National Museum Helminth. Coll. No. 55650

Paratypes: Helminthological Collection, University of California at Los Angeles, No. 4, Author's personal collection, No. 716

In the extreme disparity in length between the right and the left spicules, *C. readi* n. sp. differs from all other species of *Cyrtosomum*. In addition to the spicule size difference, *C. readi* n. sp. differs from *C. scelopori* Gedoelst, 1919, in the number and arrangement of cephalic and caudal papillae.

In the cecae of 41 of 749 specimens of North American Iguanidae examined, worms were recovered which could be assigned to the genus *Cyrtosomum* but which differed sufficiently from previously described forms to be considered a new species. The name *Cyrtosomum heynemani* n. sp. is proposed for this new species in honor of Dr. Donald Heyneman of the Department of Zoology, University of California at Los Angeles.

The earless lizard, *Holbrookia maculata* Girard, had the highest incidence of infection with this species, with 23 of 48 specimens examined being parasitized. In contrast, only 2 infections were found upon examination of 39 specimens of the greater earless lizard, *Holbrookia texana* (Troschel). Six of 13 *Sceloporus clarki*

Baird and Girard examined were infected with this species. Examination of 63 specimens of the eastern fence lizard, *Sceloporus undulatus* (Daudin), and 30 specimens of the red scaly lizard, *Sceloporus poinsettii* Baird and Girard, revealed only a single infection of *C. heynemani* n. sp. in each species of host. Of 22 *Crotaphytus collaris* Say examined, 8 were infected with *Cyrtosomum* spp. Four of the 8 had *C. heynemani* n. sp. infections. Three of the 8 had double infections, *C. heynemani* n. sp. and *C. readi* n. sp. The presence of both morphological forms in single host specimens corroborates the argument for naming 2 new species for these distinct types. In as much as the specific differences remain constant in the double infections as well as in others, breeding isolation of the 2 populations appears to have occurred.

Although limited numbers of specimens from southeastern United States were examined, the single infected *Sceloporus woodi* Stejneger found suggests a wide range across the southern United States for this parasite.

Cyrtosomum heynemani n. sp.

Diagnosis: Atractidae Travassos, 1919, with characters of the genus *Cyrtosomum* Gedoelst, 1919.

Anterior extremity rounded. Each lip with 1 papilla. Papillae on subventral and subdorsal lips extensible and terminate in a spherical knob. A medio-dorsal and a medio-ventral papilla occur.

Male: 2.25 (1.76–2.48) 2.17 long, 0.112 (0.096–0.112) 0.109 wide at level of esophageal bulb. Esophagus 0.449 (0.400–0.497) 0.450 long. Muscular anterior portion of esophagus 0.273 (0.240–0.289) 0.266, posterior portion 0.176 (0.160–0.208) 0.184 long. Ratio esophagus length to body length less esophagus length .25 (.23–.32) .27. Ratio esophageal parts .69 (.64–.73) .69. Esophageal bulb 0.072 (0.064–0.072) 0.070 long by 0.072 (0.064–0.072) 0.070 wide. Cloaca 0.080 (0.080–0.096) 0.086 from posterior extremity. Spicules unequal, sharply pointed distally. Right spicule 0.096 (0.088–0.104) 0.094 long, left spicule 0.152 (0.152–0.160) 0.155 long. Ratio right to left spicule .60 (.58–.65) .61. Ratio left spicule length to body length 0.068 (0.062–0.082) 0.072.

Seven pairs of caudal papillae: 3 subventral pre-cloacal pairs; 4 post-cloacal pairs (1 pair lateral, 2 ventral, 1 latero-dorsal).

Female: 1.90 (1.80–2.00) 1.92 long, by 0.144 (0.128–0.192) 0.160 wide at level of esophageal bulb. Esophagus 0.400 (0.352–0.432) 0.392 long. Muscular portion of esophagus 0.240 (0.224–0.240) 0.237 long, glandular portion 0.160 (0.128–0.192) 0.160 long. Ratio esophagus length to body less esophageal length .25 (.22–.29) .26. Ratio esophageal parts .67 (.57–.80) .68. Esophageal bulb 0.080 (0.064–0.080) 0.078 long by 0.088 (0.080–0.096) 0.090 wide. Anus 0.160 (0.096–0.192) 0.154 from posterior extremity. Vulva 0.128 (0.112–0.160) 0.138 from anus and 0.288 (0.228–0.352) 0.292 from posterior extremity.

Type Host: Holbrookia maculata Girard (In Herpetological Collection, University of California at Los Angeles. Coll. No. 3708)

Location: Cecum

Type Locality: Socorro County, New Mexico

Type Specimens: Holotype: Male. Allotype: Female. U. S. National Museum Helminthological Coll. No. 55651

Paratypes: Helminthological Collection, University of California, Los Angeles, No. 3. Author's personal collection, No. 151

C. heynemani n. sp. differs from *C. scelopori* Gedoelst, 1919 in the number and arrangement of cephalic and caudal papillae. *C. heynemani* is a much larger worm than the remaining species of *Cyrtosomum*. In addition, it differs from these in the relative, as well as the absolute, size of the left spicule. The ratio of the right to the left spicule also serves to differentiate *C. heynemani* n. sp. from all other species of *Cyrtosomum*.

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Explanation of Plate I

Cyrtosomum readi n. sp. from *Gambelia wislizenii silus* Stejneger.
FIGURE 1. Female head, *en face* view.
FIGURE 2. Female, whole body.
FIGURE 3. Male, whole body.
FIGURE 4. Female caudal region, lateral view.
FIGURE 5. Male caudal region, lateral view.

Explanation of Plate II

Cyrtosomum heynemani n. sp. from *Holbrookia maculata* Girard.
FIGURE 6. Female head, *en face*.
FIGURE 7. Female, whole body.
FIGURE 8. Male, whole body.
FIGURE 9. Female caudal region, lateral view.
FIGURE 10. Male caudal region, lateral view.

RESEARCH NOTE

INFECTIVITY OF EXCYSTED CYSTICERCOIDS OF *HYMENOLEPIS NANA*

Previous studies (Voge and Heyneman, 1958, J. Parasitol., **44**: 249–260) have shown the variation in development of the outer membranes of *Hymenolepis* cysticercoids grown in different environmental temperatures. It was postulated that there might be a relation between the thickness of these membranes and infectivity of the larva in the final host. Cysticercoids of *Hymenolepis nana* grown in beetles maintained at 40–42° C, have very thin and fragile membranes and are not infective, although the scoleces appear normal. An attempt was therefore made to remove the membranes of normally developed larvae by digestion and to test infectivity of the freed scoleces in the final host. All experiments were performed with the Rice Institute strain of *Hymenolepis nana* and with the flour beetle, *Tribolium confusum*, maintained in enriched flour. A parasite-free strain of Swiss White mice from Rockland Farms, New City, New York, was used. Careful checks were made for the presence of helminths before each experiment. Mature cysticercoids grown at 30° C were exposed to 100,000 Armour units of tryptic activity (Tryptar, Armour Laboratories) in M/15 phosphate buffer, pH 7.2 made isotonic with NaCl. Exposure was for 30–90 minutes at 37° C, in a Dubnoff metabolic shaker. Careful observation was maintained to ensure that scoleces were entirely freed from all vestiges of their cysticercoid membranes. The only remaining sign of these structures was a knot of tissue adhering occasionally on the terminal portion of the cysticercoid tail. In the first series, 8 mice were fed 10 excysted cysticercoids each. Eight control mice were given the same number of undigested cysticercoids kept at 37° C in saline for the same period. At autopsy 10 days later, 50% of the test mice harbored adult worms, and a total of 10% of the scoleces fed were recovered; 100% of the control mice were positive and 40% of the worms were recovered. In another series, cysticercoids were digested as before and each of 8 mice fed 4 cysticercoids. Only 1 worm was recovered at autopsy. In the third experiment 12 mice were fed 10 digested cysticercoids each. Four of the 12 mice harbored worms at autopsy, and 9 of the 120 worms fed (7.5%) were recovered.

From these preliminary observations it appears that the membranes which envelop the cysticercoid of *H. nana* are not essential for protection against the final host's digestive enzymes. The young scolex of *H. nana* may possess a relatively high degree of resistance to digestion, rendering the membranes perhaps less essential for survival of this species in the warm blooded host, than for related forms such as *H. diminuta*.—D. HEYNEMAN, J. F. WELSH AND M. VOGE, *Departments of Zoology and Infectious Diseases, University of California, Los Angeles.*

PLATE I

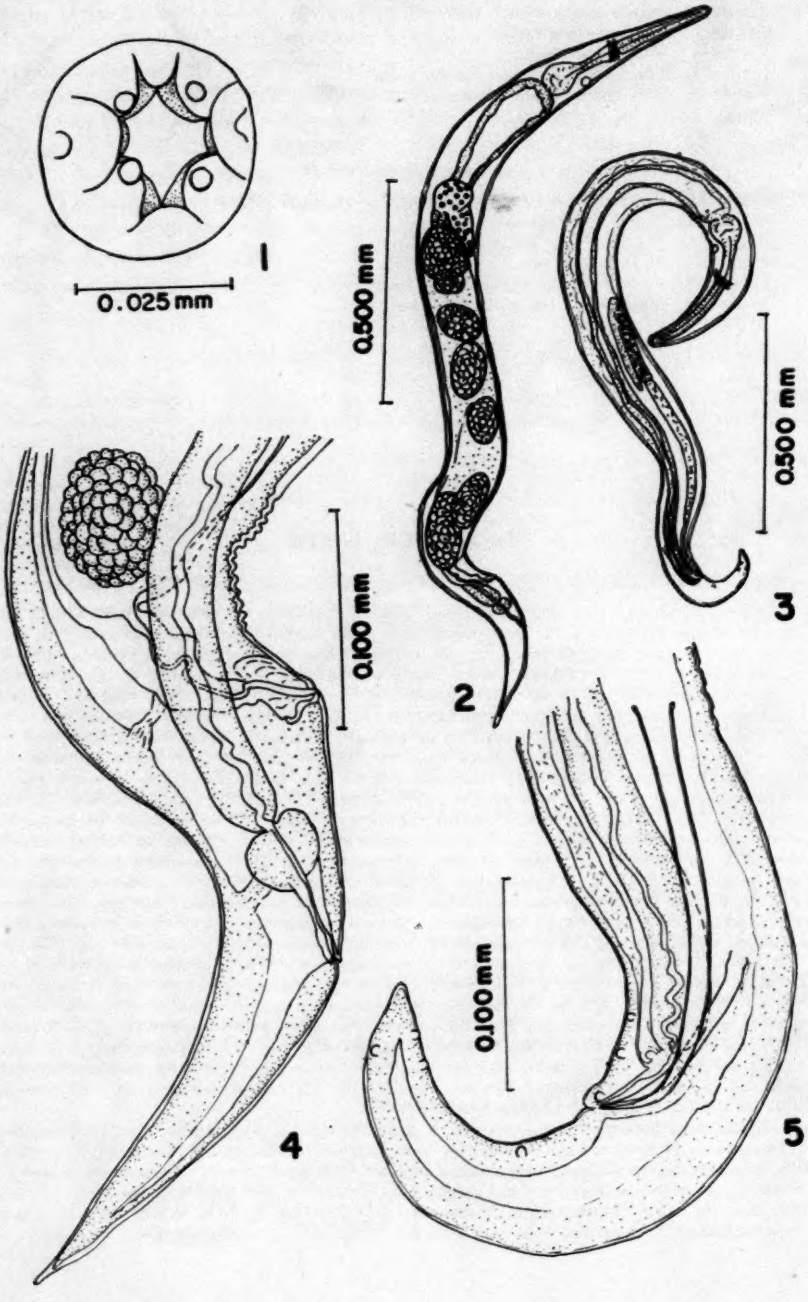
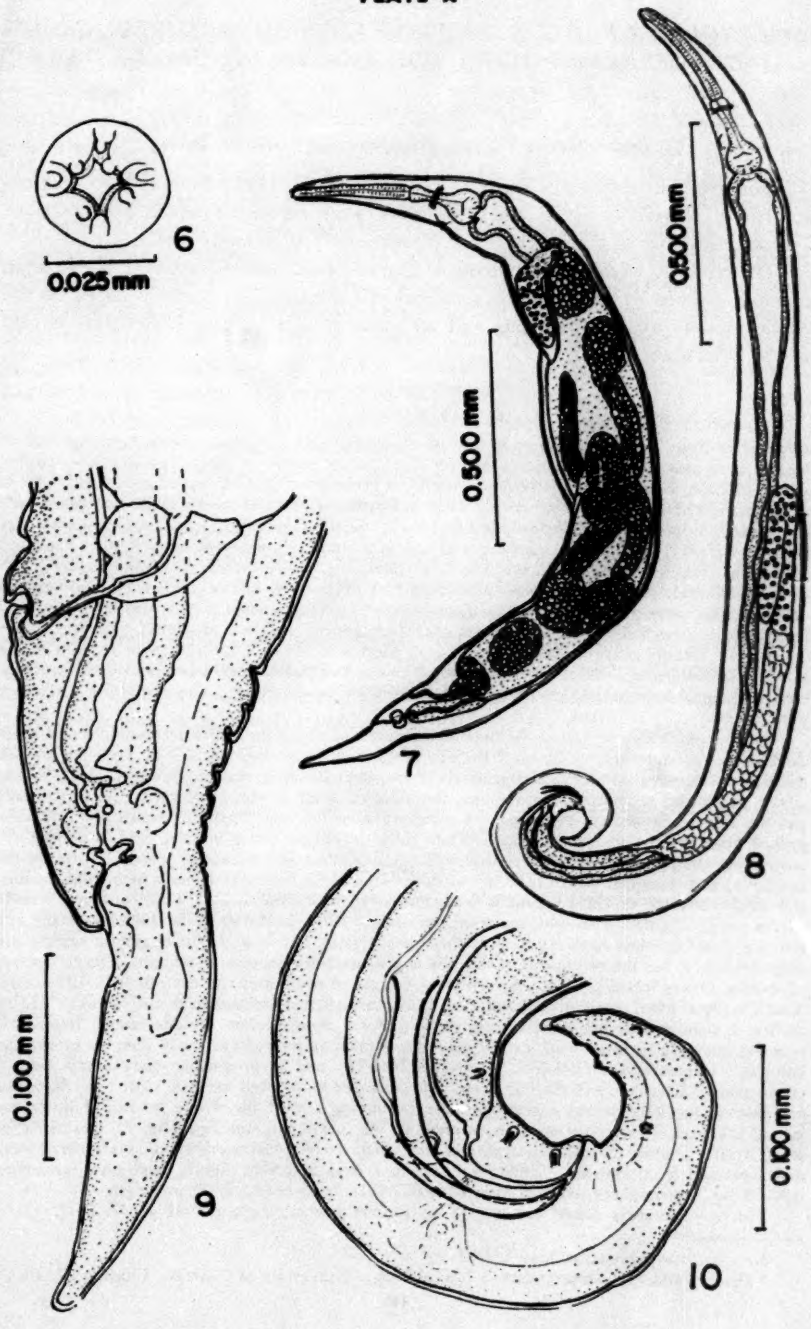


PLATE II



HYMENOLEPIS BAHLI N. SP. FROM GREY MUSK SHREW, *CROCIDURA CAERULEA* (KERR, 1792) PETERS, 1870 FROM INDIA

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One grey musk shrew, *Crocidura caerulea* (Kerr, 1792) Peters, 1870 was examined in November, 1955 and 2 cestodes were recovered which are described here as belonging to a new species of *Hymenolepis* Weinland, 1858.

The cestodes were fixed in alcoholic Bouin's fluid and stained with acetic alum carmine, cleared in terpineol and mounted in balsam. This study is based on the whole mounts of the specimens and all measurements are in millimeters except where otherwise stated.

Hymenolepis bahli n. sp.
(Figs. 1–7)

Diagnosis: Two strobila measure 38 and 41 in length and additional pieces totalling 12.5 in length were also found. Maximum breadth 0.91–1.02 in region of gravid proglottids. Scolex small, globose, 0.30–0.32 in diameter. Rostellum introverted in both specimens, 0.047–0.05 in diameter, with 1 row of 10 small hooks, 0.018 in length. Handle of hooks small and guard well developed. Rostellar pouch 0.18–0.184 × 0.11–0.12. Suckers rounded, shallow and slightly developed, 0.09–0.11 in diameter. Scolex narrows to a short unsegmented neck, 0.16 in diameter. Numerous immature proglottids, much broader than long, and posterior of these measure 0.12 × 0.36. Proglottids protandrous, testes developing first followed by cirrus pouch and female genital organs. Numerous mature proglottids, much broader than long, anterior ones measure 0.13 × 0.42 and posterior ones 0.20 × 0.70. Numerous gravid proglottids and last proglottid measures 0.037–0.51 × 0.91. Genital pores unilateral, present on right side, 0.1–0.11 from anterior margin which is either in middle or a little posterior to it. Ventral longitudinal excretory duct 0.015 in diameter and dorsal longitudinal excretory duct 0.004–0.006 in diameter. The transverse excretory duct not seen.

Testes rounded structures close together in middle of proglottid. One testis anterior to other 2 which are at same level. In early mature proglottids testes measure 0.1 in diameter and all testes are of same size. With the gradual development of other organs, however, specially the cirrus pouch and receptaculum seminalis, the anterior testis is pushed towards the aporal side and the testes now occupy the angles of a right-angled triangle. Posterior testes are gradually pushed apart by the growing ovary. When fully developed testes become oval being anteroposteriorly compressed and measure 0.09–0.16 × 0.12 Cirrus pouch extends about 2/7 across the proglottid and measures 0.14–0.17 (0.16) × 0.03–0.04 (0.034). External vesicula seminalis rounded and small; internal vesicula seminalis well developed, occupying almost whole of cirrus pouch. Cirrus small, slightly developed, unarmed, measuring 0.016–0.019 (0.018) in length. Ovary and vitelline gland are last to develop, and when they appear, not only the male genital organs are fully developed, but the receptaculum seminis is also well developed and contains a large number of sperms. Ovary lobed, present near posterior margin of proglottid and extends 0.23–0.31 across. Vitelline gland lobed, present just posterior to ovarian isthmus and measures 0.07 across. Vagina at first a slender tube but dilates quite early to form receptaculum seminis, which initially is rounded but soon becomes oval, extending from poral longitudinal excretory duct up to ovarian isthmus. It measures 0.13–0.29 (0.19) × 0.08–0.16 (0.11) and is present on the ventral side of cirrus pouch. Uterus arises directly from ovarian lobes as a lobed sac but soon extends dorsal and beyond the longitudinal excretory ducts, occupying almost the whole proglottid up to the lateral margins. Numerous eggs are present in the uterus and the egg (fig. 7) was sketched when fresh. Chorion smooth and oval, measuring 0.05 × 0.04. Extra-embryonic blastoderm wavy and separated from chorion. Embryonic envelope oval and very lightly papillated measuring 0.04 × 0.04. Onchosphere oval or rounded, 0.03 × 0.03 the 6 hooks 0.016 in length.

The new species is named in honor of my former teacher, the late Prof. K. N. Bahl.

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DISCUSSION

There are about 425 valid species of the genus *Hymenolepis* Weinland, 1858. Of these about 125 possess 10 rostellar hooks, but only the following species have been recorded from Insectivora: *H. jacobsoni* v. Linstow, 1907 from Java; *H. petrodromi* Baer, 1933 from Rhodesia; *H. scutigera* (Dujardin, 1845) Meggitt, 1924 from Europe; *H. singularis* Cholodkovsky, 1913 from Europe; *H. parvissima* Voge, 1953 from U.S.A.; *H. sphenomorphus* Lucker & Rausch, 1952 from U.S.A.; *H. blarinae* Rausch & Kuns, 1950 from U.S.A.; *H. parva* Rausch & Kuns, 1950 from U.S.A.; *H. virilis* Voge, 1955 from U.S.A.; *H. lineola* Oswald, 1951 (= *H. parva* according to Senger, 1955) from U.S.A.; and *H. longi* Oswald, 1951 from U.S.A. Besides these, there are some 25 species of *Hymenolepis* described from Insectivora, but insofar as is known to the author only 4, *H. bacillaris* (Goeze, 1782) Blanchard, 1891; *H. furcata* (Stieda, 1862) Blanchard, 1891; *H. solitaria* (Meggitt, 1927) Hubscher, 1937 and *H. minutissima* (Meggitt, 1927) have been described from Rangoon, Burma and none from India.

The present species can be separated from the closely related species thus:

H. jacobsoni has comparatively small rostellum (0.039) but longer hooks (0.021–0.023). *H. petrodromi* has comparatively small hooks (0.011–0.013). *H. scutigera* has comparatively large rostellar hooks (0.033–0.04) of a different shape; the uterus is arc-shaped and the gravid proglottids are greatly elongated. *H. singularis* has very large rostellar hooks (0.061–0.062). *H. parvissima* has a very small strobila (0.467–0.85) with only a few proglottids (7–10) and the testes are in a straight line. *H. sphenomorphus* has the testes in a line, the cirrus with a well developed armed knob, and a small strobila with 22 proglottids. *H. blarinae* has a long strobila (90) but a small scolex (0.25), the rostellar hooks are longer (0.033) and the cirrus is armed. *H. parva* has a small strobila (3–5) with a small scolex (0.16–0.19) with large rostellar hooks (0.03–0.044) of a different shape; and the testes are in a line. *H. virilis* has a small strobila (1.0–2.1) but large rostellar hooks (0.028–0.032); the cirrus is armed. *H. longi* has a small strobila (0.75–1.25) and only a few proglottids are present; the cirrus is armed and the uterus reticulate.

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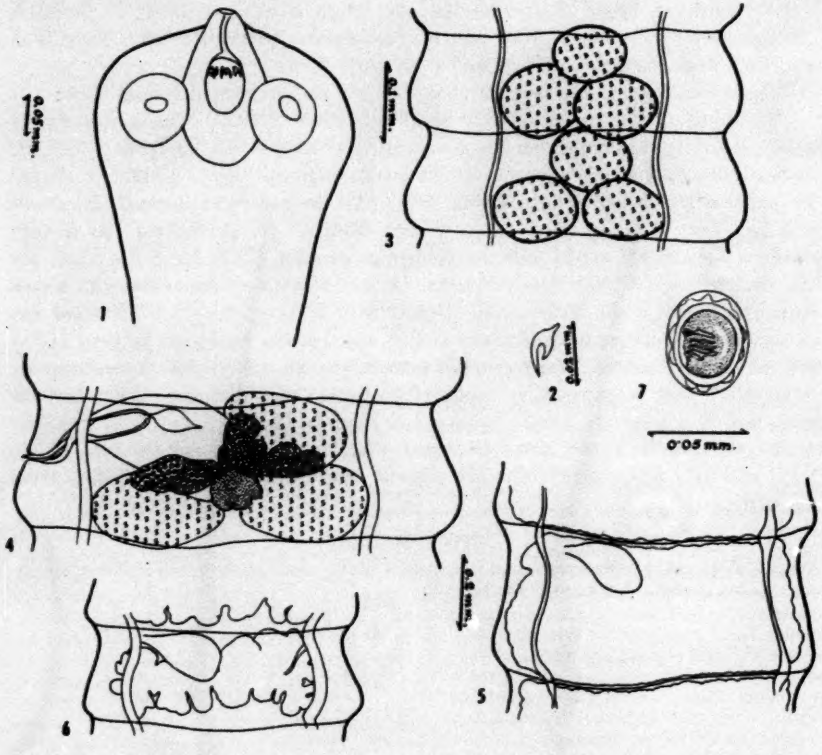
EXPLANATION TO FIGURES

(All diagrams made with the aid of camera lucida.)

Hymenolepis bahli n.sp.

FIGURE 1. Scolex. FIGURE 2. Rostellar hook. FIGURE 3. Proglottid with testes. FIGURE 4. Mature proglottid. FIGURE 5. Early gravid proglottid. FIGURE 6. Late gravid proglottid. FIGURE 7. Egg.



TRANSVERSOTREMA LARUEI, A NEW TREMATODE OF PHILIPPINE FISH (DIGENEA: TRANSVERSOTREMATIDAE)*

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Ten specimens of *Lates calcarifer* Bloch commonly called *apahap* (Tagalog) bought from Central and Quiapo markets, Manila, Philippines, were examined for helminth infection. This species, also known as sea bass, is a marine fish which comes into brackish and fresh waters. The specimens were taken from brackish water fish ponds at Malabon, Rizal Province.

One fish was infected with 3 metacercariae, 1 in muscle and 2 in the intestine.

The new species herein described as *Transversotrema laruei* n. sp. has the characteristics of a fully developed worm except for the absence of eggs in the uterus. It resembles *Cercaria koliensis* Olivier.

Additional information as to the life cycle of *Transversotrema*, the fish host, organs infected and distribution is herein reported.

Morphological diagnosis was made from specimens fixed in Bouin's picro-formol under cover slip. They were kept for 24 hrs in the fixative and later stored in 70% glycerine-alcohol. The worms were stained in aceto-carmine of about 4:1 dilution.

Transversotrema laruei n. sp.
(Figs. 1 and 2)

Specific diagnosis: Body broad, leaf-like and somewhat indented at the mid-posterior end. Length 0.2–0.26; width 0.39–0.4 mm. Eye spots large, posterior to the pharynx and a little anterior to acetabulum. Ventral surface of body covered with large, conspicuous, closely set, plate-like spines diagonally arranged. Dorsal surface with spines on anterior portion, perhaps less abundant than on ventral side. Acetabulum, disc-shape, diameter 0.06–0.09 mm. Oral sucker absent. Mouth opening directly into pharynx of 0.03–0.035 mm in diameter. Esophagus short and narrow, bifurcating at level of acetabulum, intestinal branches united posteriorly, thus forming an intestinal loop surrounding reproductive organs. Intestinal wall slightly diverticulate in 1 specimen but less in others. Testes lobed, within intestinal loop and bilateral. Ovary left, anterior to testis but a little smaller. Oviduct short, conical. Laurer's canal? Uterus well formed with transverse part passing over testis immediately posterior to intestine and then ascending into common genital sinus, genital pore median marginal, anterior to mouth. Vas deferens narrow, coiled just anterior to intestinal loop, then ascending and opening together with the uterus into common genital sinus. Copulatory organs absent. Vitellaria follicular, extra-intestinal, with prominent mid-anterior and posterior gaps. Excretory vesicle not seen, some vessels in anterior and lateral fields.

Host: Lates calcarifer Bloch.
Locality: Malabon, Rizal, Philippines.
Organs: Muscles and intestine.
Type: U.S.N.M. Helm. Coll. No. 38296.
Paratypes: Dept. of Zoology, University of the Philippines, Helm. Coll. Nos. 451f and 576f.

Cercaria patialensis Soparkar, 1924 from India and later reported in the Congo

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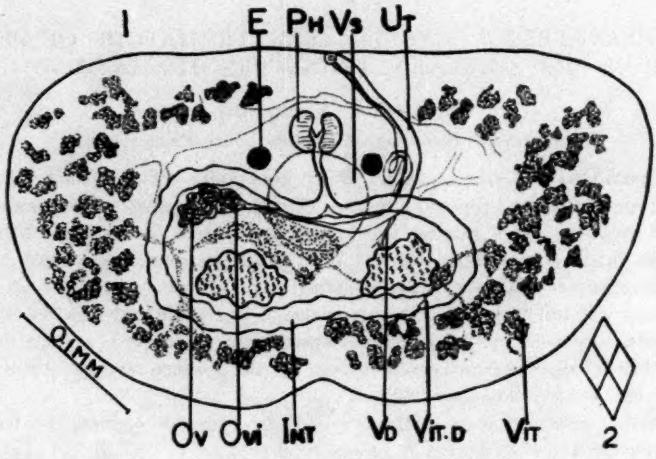
* This investigation was made while the author was a Guggenheim Fellow and University of the Philippines Fellow. Collections of material were made through a grant-in-aid of the Natural Science Research Center, University of the Philippines.

The author is greatly indebted to Dr. George R. LaRue, Animal Parasite Laboratory, Animal Disease and Parasite Research Division, Agricultural Research Center, Beltsville, Maryland for the use of his laboratory and his very valuable criticisms and suggestions; to Mr. Allen McIntosh of the same institution who so very kindly offered his assistance in many ways; and to Dr. George R. Wharton, Head, Department of Zoology, University of Maryland for his kindness in providing the necessary laboratory facilities.



EXPLANATION OF FIGURES

FIGURE 1. *Transversotrema laruei* in dorsal aspect. Abbreviations: E, eye; Ph, pharynx; Vs, ventral sucker; Ut, uterus; Ov, ovary; Ovi, oviduct; Int, intestine; Vd, vas deferens; Vit.d, vitelline; Vit, vitellaria.

FIGURE 2. Diagrammatic drawing showing the shape and arrangement of spines from the ventral side.

by Brien, 1954 and *Cercaria koliensis* Olivier, 1947 from Guadalcanal, Solomon Islands seem to be the only 2 cercariae reported which may be referred to the family Transversotrematidae Yamaguti, 1953. *Transversotrema haasi* Witenberg, 1944 reported from the Red Sea is the only adult ever described but with no record of host or organ. The comparative measurements of all known species belonging to the above named family are shown in Table I. The pharynx in the cercariae is designated by Soparkar and Olivier as oral sucker; however, Witenberg correctly called it a pharynx.

Transversotrema haasi and *T. laruei* n. sp. are congeneric but differ as to size, shape of body, arrangement of vitellaria, relative proportions of pharynx and acetabulum and shape of spines. However, in shape of body and arrangement of organs *T. laruei*, a metacercaria, presents some resemblances to *Cercaria koliensis*. However, its body is smaller, and it differs as to relative proportions of pharynx and acetabulum, and as to shape of spines. In all measurements it is much smaller than *C. patialensis*. In view of these differences in size relations and differences in the spines, *T. laruei* must be considered to be a new species.

TABLE I. *Comparative measurements of known species of Transversotrema in mm (averages in parentheses).*

	C. patialensis Soparkar 1924	*C. koliensis* Olivier 1947	*T. haasi* Witenberg 1944	*T. laruei* n. sp
Length	0.374–0.459	0.24 –0.31 (0.27)	0.8–1.25	0.2 –0.26
Width	0.697–0.782	0.26 –0.49 (0.35)	2.5–4.0	0.39–0.4
Diameter of Pharynx	0.058–0.078	0.045–0.055 (0.05)	0.2	0.03–0.035
Diameter of Acetabulum	0.136	0.095–0.110		0.06–0.09

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RESEARCH NOTES

THE SUSCEPTIBILITY OF TWO STRAINS OF WHITE MICE TO *SYPHACIA OBVELATA* INFECTIONS

For several years, we have been using a uniform strain of white mice from our nematode-free mouse colony for both our biological and chemotherapeutic studies on *S. obvelata*. Because of difficulties encountered in infecting white mice from other sources, it occurred to us that there may be variations in the susceptibility to *S. obvelata* among strains of white mice. We, therefore undertook a comparative study employing mice from our colony, henceforth designated the "Columbia strain," and the CF1 strain obtained from Carworth Farms. The latter mice were first cleared of oxyurids and were raised and bred for several months in accordance with our standard procedures for the maintainence of nematode-free mouse colonies before they were used in this study. (Chan, 1952, Am. J. Hyg. 56: 14–21.)

In Experiment 1, equal numbers of male and female mice 3–4 weeks old and weighing between 16–20 grams, from each of the two colonies were employed. The animals were given 100 *Syphacia* eggs by stomach tube on 3 occasions at 3-day intervals and were killed 3 days after the last dosing. Each mouse thus harbored worms in 3 different stages of development, namely 9-day, 6-day and 3-day stages respectively. The worms were enumerated on the basis of their sizes which indicated their age. Only female worms were included in the tabulations as *Syphacia* males mature in 6 days and normally then pass out of the hosts.

In Experiment 2, male mice of similar age and weight of both mouse strains were fed 200 *Syphacia* eggs on 2 occasions and 6 days apart. These animals were sacrificed 3 days after the last dosing and harbored 9-day and 3-day worms which were similarly enumerated.

Table I summarizes the results of Experiments 1 and 2. It is apparent from the data that mice of the Columbia strain are more susceptible to *S. obvelata* than are mice of the CF1 strain. In Experiment 1, the average number of worms harbored by the Columbia mice was 70.7 per animal, whereas the worm-burden of the CF1 strain averaged only 42. While the differences in the total worm-burden among these mice were noteworthy, only the 9-day stage of the worms showed a statistically significant difference; the Columbia mice harbored an average of 32.2 at this time, while the Carworth Farm mice harbored only 9.5. There were insignificant differences in the worm-burden of the 6-day and 3-day old *Syphacia* in the two strains of mice. In Experiment 2 with increased egg-dose, the pattern appeared similar. The Columbia mouse series averaged 81.5 worms while the CF1 group had an average of 35.7 worms. Again the number of mature *Syphacia* harbored by the mice differed markedly according to strain; the Columbia mice harbored 52.3 of the 9-day stage compared with 8.8 worms per CF1 mouse, the differences being statistically significant. The former mice also harbored an average of 29 immature *Syphacia* and the latter mice an average of 26.8 of similar stages.

These results suggest that strains of mice vary in their resistance to *S. obvelata* and that this resistance may be partly explained by the failure of worms to mature beyond certain stages of development. It appears then that fewer worms are able to reach maturity in the CF1 mice than in the Columbia strain. It is possible, of course, that mature *Syphacia* worms may be further reduced after 9 days and prior to the onset of migration of the gravid worms. Inasmuch as the method usually employed for the infection of mice with *S. obvelata* (Chan, 1952, Am. J. Hyg. 56: 22–30) is dependent upon the extent of contamination of the environment by the migration of gravid worms, this strain resistance of mice is of special importance in experimental studies on this parasite. Mice of the *Syphacia*-resistant strain when used as infected "source" animals for infecting negative animals would be ineffective in producing satisfactory quantitative infections because of the small number of maturing worms. More importantly these mice would be unable to maintain their own heavy infections as fewer migrating worms would shed fewer eggs for re-infection purposes.

Any influence of sex (Mathies, 1954, J. Parasitol. **40**: 702) on *S. obvelata* infections in mice was evident against mature worms only. In Experiment 1, although the total worm-burdens of the male and female mice showed negligible differences, there was at least 50% more 9-day worms in the male mice than in the female mice. This effect may also be explained in part by the failure of *Syphacia* worms in the female hosts to attain maturation, the resistance operating in a similar manner to the strain resistance of mice to this pinworm.—K. F. CHAN AND SYDELL KOPILOF, *School of Public Health and Administrative Medicine, Columbia University.* (Present addresses: Mount Morris State Hospital, Mt. Morris, N. Y. and Division of Parasitology, Cornell University Medical College, New York City.)

TABLE I. *Number of worms recovered from Columbia and CF1 mice following infection with equal numbers of eggs of* Syphacia obvelata.

Mouse		Worm Burden and Stages							
		Columbia Strain				CF1 Strain			
No.	Sex	9-day (4.4–4.9 mm)	6-day (2.6–3.8 mm)	3-day (1.12 mm)	TOTAL	9-day (4.4–4.9 mm)	6-day (2.6–3.8 mm)	3-day (1.12 mm)	TOTAL
Experiment 1									
1	Male	76	6	5	87	4	12	2	18
2	Male	14	36	38	88	7	11	15	33
3	Male	50	12	7	69	15	7	17	39
4	Male	52	11	0	63	23	23	8	54
5	Male	24	9	17	50	20	50	20	90
AVERAGE		43.2	14.8	13.4	71.4	13.8	20.6	10.4	46.8
1	Female	6	13	26	45	5	19	40	64
2	Female	10	6	28	44	3	3	2	8
3	Female	32	28	13	73	4	4	6	14
4	Female	44	27	56	127	5	6	5	16
5	Female	14	37	10	61	9	57	18	84
AVERAGE		21.2	22.2	26.6	70	5.2	17.8	14.2	37.2
COMBINED AVERAGE		32.2	18.5	20	70.7	9.5	19.2	12.3	42
Experiment 2									
1	Male	53		12	65	10		29	39
2	Male	45		41	86	14		5	19
3	Male	39		52	91	4		39	43
4	Male	38		15	53	14		2	16
5	Male	65		34	99	3		41	44
6	Male	74		21	95	8		45	53
AVERAGE		52.3		29	81.5	8.8		26.8	35.7

RECORD OF THE PARASITIC COPEPOD *LERNAEA CYPRINACEA* L. IN OREGON AND WASHINGTON FISHES

In July, 1957 the Oregon Game Commission was called upon to investigate a sudden epizootic among yearling rainbow trout (*Salmo gairdnerii*) in a small spring-fed pond at a private trout-fishing establishment near Tigard, Oregon. Mature lernaeid copepods were observed on all of several hundred trout examined. Some of the fish were so heavily parasitized that one or both eyes were destroyed. As the pond effluent drained into a nearby tributary of the Willamette River, an important trout and salmon habitat, it was deemed necessary to destroy the entire stock of fish. Representative specimen material was subsequently identified as *Lernaea cyprinacea* Linnaeus, 1758 (= *L. elegans* Leigh-Sharpe, 1927; = *L. carassii* Tidd, 1933).

Additional specimen material representing *L. cyprinacea* from Oregon was kindly donated for study by Dr. James E. Lynch, College of Fisheries, University of Washington. The material consisted of 4 adult females from a 113-mm juvenile king salmon (*Oncorhynchus tshawytscha*) taken at the mouth of Oswego Creek, Oswego, Oregon; supporting data indicated that the host was 1 of 6 similarly parasitized salmon from an original collection of 9 taken August 17, 1948.

The occurrence of *Lernaea* in Washington waters was recently established with identification of *L. cyprinacea* from 16 hosts representing 8 species of cyprinid and centrarchid fishes (Table I) from the lower Yakima River, a south-central Washington tributary of the Columbia River. These fish were brought to the attention of the senior author following an extensive survey of resident fishes of that area by R. Thompson and B. Patten of the U. S. Fish and Wildlife Service. We are indebted to these workers for their cooperation and for assistance with specific identification of certain of the hosts.

TABLE I. *Record of L. cyprinacea in Oregon and Washington fishes.*

Host	*Locality*	*Collection Date*
King salmon* (*Oncorhynchus tshawytscha*)	Oswego, Oregon	August 17, 1948
Rainbow trout* (*Salmo gairdneri*)	Tigard, Oregon	July 25, 1957
Chiselmouth* (*Acrocheilus alutaceus*)	Yakima River, Wash.	October, 1957
Columbia squawfish* (*Ptychocheilus oregonensis*)	"	"
Black-sided dace* (*Rhinichthys oculus*)	"	"
Long-nosed dace* (*Rhinichthys cataractae*)	"	"
Carp (*Cyprinus carpio*)	"	"
Smallmouth black bass (*Micropterus dolomieu*)	"	"
Largemouth black bass (*Micropterus salmoides*)	"	"
Pumpkinseed (*Lepomis gibbosus*)	"	"

* New host record.

Various species of *Lernaea* have been reported previously from a variety of fresh-water fishes in eastern and central United States. Haderlie (1953, Cal. Pub. Zool. **57**: 303–439) extended the known range of the genus to the Pacific slope with records of *L. carassii* (= *L. cyprinacea*) from 15 species of Northern California fishes. The present records extend the known range of *Lernaea*, specifically *L. cyprinacea*, to the Columbia River Basin. It is interesting to note that Bangham and Adams (1954, J. Fish. Res. Bd. Canada **11**: 673–708) conducted an extensive survey of the parasites of British Columbia fishes, including 11 of 17 host genera cited here, or by Haderlie, but did not encounter any representative of *Lernaea*. It is possible that the present records approximate the northwestern range of the genus in North America. The occurrence of *Lernaea* in the salmonid fishes *O. tshawytscha* and *S. gairdnerii* constitutes an extension of the known host range to the Family Salmonidae.

Several of the hosts cited in Table I, namely, *C. carpio*, *M. dolomieu*, *M. salmoides*, and *L. gibbosus*, are not native to Pacific drainages, but were directly introduced at various times in the past when empirical stocking of exotic species was a popular, albeit misguided, practice. It is probable that *L. cyprinacea*, and perhaps other species of *Lernaea* as well, entered the waters of the Pacific slope with one or more of these alien species.

Lernaeid copepods have figured elsewhere in fish mortalities, particularly in goldfish (*Carassius auratus*) and carp (*C. carpio*) under conditions of high population density and high temperature. Those concerned with the propagation and conservation of fishes should be aware of this potential, and exercise extreme caution in transplantation of susceptible stocks of fishes from *Lernaea*-endemic areas to localities known or presumed to be *Lernaea*-free.—J. R. UZMANN and H. J. RAYNER, *U. S. Fish and Wildlife Service, Seattle, Washington* (J.R.U.), *and Oregon State Game Commission, Portland* (H.J.R.).

RECORD OF THE FUNGUS *ICHTHYOSPORIDIUM* CAULLERY AND MESNIL, 1905, IN IDAHO

In September, 1957, an organism identified as a member of the genus *Ichthyosporidium* Caullery and Mesnil, 1905 (= Ichthyophonus Plehn and Mulsow, 1911) was observed in wet mounts and stained tissue sections of material from the kidney and liver of Rainbow Trout (*Salmo gairdnerii*) received by this laboratory from hatcheries adjacent to the Snake River in south-central Idaho.

Fantham and Porter (1948, Proc. Zool. Soc. London **117**: 609–649) reporting on an Eastern Brook Trout (*Salvelinus fontinalis*) taken in Grand Lake Long, Province of Quebec, in 1933 and 1935, described a parasite which they considered to be *Ichthyosporidium* sp. but retained the genus in the Haplosporidia without further comment. Rucker and Gustafson (1953, Prog. Fish Cult. **15**: 179–181) reported an ichthyosporidiosis epizootic among Rainbow Trout from 3 localities in western Washington. In subsequent studies Gustafson and Rucker (1956, Spec. Sci. Rep't. Fisheries No. **166**, U. S. Department of the Interior) stated that the organism observed by them appeared to be generally like *I. hoferi*. We consider the organism under investigation in our laboratory to be identical to the one described by Rucker and Gustafson and by Robertson (1909, Proc. Zool. Soc. London **1**: 399–402).

Livers from the diseased fish were grossly normal in color or lacked the usual dark red coloration and varied from pale red to yellow-tan. Multiple yellow-white lesions were seen in many of the abnormally colored livers. Kidneys of all fish examined were swollen, with irregular surfaces, and presented a grayish, mottled appearance. A viscous, blue-gray fluid was released when the membrane of this organ was ruptured.

The organisms were spherical, 10–145 microns in diameter, granular, opaque to translucent, and had relatively heavy walls. The germinating spheres had extruded single to multiple, unbranched or branched germ tubes. The cytoplasm of a few spheres enclosed numerous refractile

bodies. The number of spheres in wet mounts of material from both liver and kidney examined under low-powered magnification was enormous. Figures 1 through 3 are photomicragraphs of *Ichthyosporidium* sp. in material from Rainbow Trout (*S. gairdnerii*) with magnification of 100×.

When material was dried and Gram-stained for routine bacterial examination, it was difficult to identify the spheres. No other organisms were identified in either wet mounts or stained preparations.

The finding of the fungus *Ichthyosporidium* Caullery and Mesnil, 1905, in Rainbow Trout (*Salmo gairdnerii*) in the Snake River area of south-central Idaho constitutes a new geographical record for the organism.—A. JOHN ROSS AND THOMAS J. PARISOT, *U. S. Fish and Wildlife Service, Seattle, Washington.*

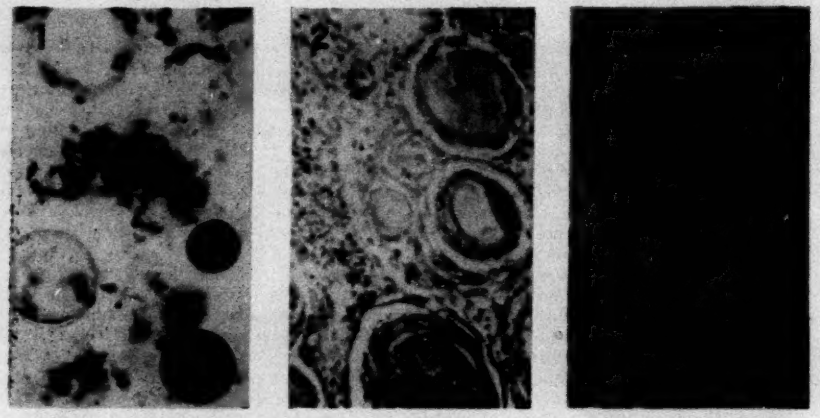


FIGURE 1. Wet mount of material from formalin preserved kidney indicating variation in size of spheres.

FIGURE 2. Hematoxylin and eosin stained section of liver showing variation of size and the heavy wall of the spheres.

FIGURE 3. Hematoxylin and eosin stained section of liver. Note multiple germ tubes extruded from the sphere.

SPHAERITA IN CYSTS OF *ENTAMOEBA COLI*

The occurrence of *Sphaerita* in free-living and parasitic protozoa has been noted in the past by many workers. A review of the subject is given by Kirby (1941, *in* Calkins and Summers, "Protozoa in Biological Research"). Dobell (1943, Parasitol. **35**: 134–185) describes *Sphaerita* in *Endolimax nana*. To our knowledge, *Sphaerita* thus far has been observed only in trophozoites of various protozoa but never in cysts.

Examination of a collection of slides from human stools containing cysts of *Entamoeba coli* stained with iron hematoxylin revealed the presence of *Sphaerita* in approximately 2 dozen cysts. No trophozoites of *E. coli* were found. The cysts were normal in appearance, most with 8 nuclei and containing varying amounts of chromatoidal material. *Sphaerita* was found in 8-nucleate cysts, in 7-nucleate cysts (Fig. 2), in 6-nucleate and in bi-nucleate cysts (Fig. 1). Two sporangia per cyst were seen twice (Fig. 1). The number of spores in different cysts varied from 4 to 20. In some, the sporangial wall was intact and well defined, enclosing approximately 12 ovoidal spores (Fig. 1). In others, the wall was either poorly defined or not observable. Several cysts contained an irregular aggregate of deeply staining spherical spores (Fig. 2). While the appearance of infected cysts was usually normal, 2 cysts were found in which the cytoplasm had degenerated so that only the cyst outline and the somewhat shrivelled nuclei could be seen in addition to many spores of *Sphaerita*.

In view of the variability in the appearance and number of spores in infected cysts, it seems probable that (1) *Sphaerita* spores are not always discarded from trophozoites before encystment, (2) *Sphaerita* may continue development within the cysts and, perhaps (3) upon excystment they may continue development in the new trophozoites.—MARIETTA VOGE AND JOHN F. KESSEL, *Department of Infectious Diseases, School of Medicine, University of California, Los Angeles.*

FIGURE 1. Microphotograph of binucleate cyst of *Entamoeba coli* showing two sporangia of *Sphaerita* and outlines of two nuclei.

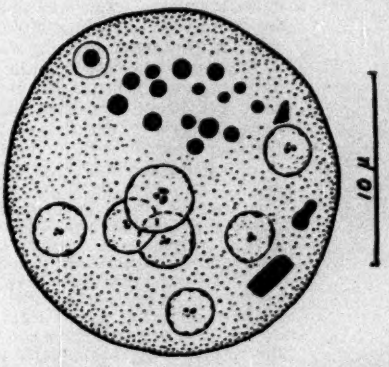
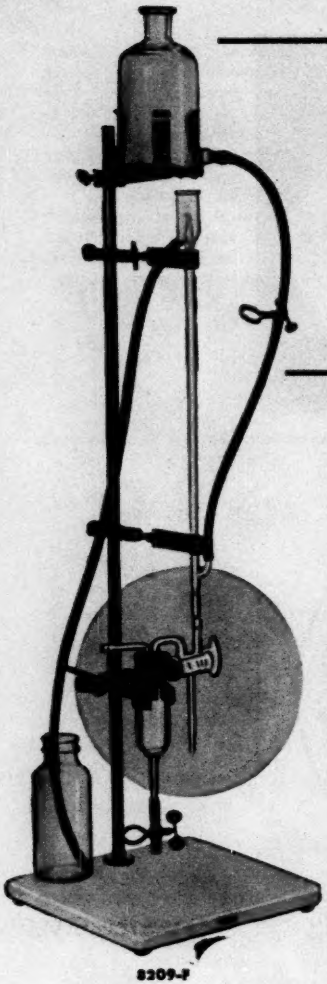


FIGURE 2. Camera lucida drawing of *Entamoeba coli* showing aggregate of spherical spores.

Table of Contents (*continued*)

RESEARCH NOTES:

AFRICAN *Hyalomma* TICK ON A MIGRANT BIRD IN EGYPT: **Makram N. Kaiser and Harry Hoogstraal, 392.** FAILURE OF THE EXCRETORY DUCTS OF A TAPEWORM (*Hymenolepis diminuta*) TO TRANSPORT ABSORBED SUBSTANCES FROM ONE PORTION OF THE WORM TO ANOTHER: **Mary Macpherson, 438.** INFECTIVITY OF EXCYSTED CYSTICERCOIDS OF *Hymenolepis nana:* **D. Heyneman, J. F. Welsh, and M. Voge, 443.** THE SUSCEPTIBILITY OF TWO STRAINS OF WHITE MICE TO *Syphacia obvelata* INFECTIONS: **K. F. Chan and Sydell Kopilof, 451.** RECORD OF THE PARASITIC COPEPOD *Lernaea cyprinacea* L. IN OREGON AND WASHINGTON FISHES: **J. R. Uzmann and H. J. Rayner, 452.** RECORD OF THE FUNGUS *Icthyosporidium* CAULLERY AND MESNIL, 1905, IN IDAHO: **A. John Ross and Thomas J. Parisot, 453.** *Sphaerita* IN CYSTS OF *Entamoeba coli:* **Marietta Voge and John F. Kessel, 454.**



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